

The
Life History of Griffithsia Bornetiana

BY



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A Dissertation submitted to the Board of University Studies
of the Johns Hopkins University in conformity with
the requirements for the degree of
Doctor of Philosophy.

1908

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With Plates XLIX-LIII, and two Figures in the Text.

THE red alga, *Griffithsia Bornetiana*, was first described by W. G. Farlow (28). It has been reported as occurring commonly from northern Massachusetts (Collins, 18, p. 50) south to Long Island Sound, and has been recorded from New Jersey (Britton, 13).² It forms rosy tufts of branching filaments (Pl. XLIX, Fig. 1), 2.5 to 15 centimetres high, on rocks, 'wharves, sponges, shells, and occasionally on *Zostera*' (Farlow, 29, p. 131). South of Cape Cod it is found growing from one to four feet below low water mark, in protected situations such as the Little Harbor at Wood's Hole, Mass., and on rocks in more exposed localities.

The present investigation was begun in 1905 on material collected at Cold Spring Harbor, New York, by D. S. Johnson in 1902, and has been continued during 1906 and 1907 at Wood's Hole and at the Johns Hopkins University.

In all plants examined, with two exceptions noted below, the antheridia cystocarps, and tetraspores are borne on separate individuals, which may be readily distinguished with the aid of a hand lens. The male plant is smaller and more compact than either the female or tetrasporic plant, and may be identified by the abrupt terminations of the filaments (Fig. 2). It rarely becomes more than 4 centimetres high. The female plant is more loosely tufted than the male, and reaches a much larger size, becoming 12 to 15 centimetres high. The cystocarps form deep red dots at the sides of the nodes (Fig. 3). The filaments of the female plant do not end abruptly, but become gradually smaller toward their tips. The tetrasporic plant is more slender than the female, and to the eye more nearly like it than the male. It may be distinguished by the whorls of tetraspores, which form complete rings at the nodes (Fig. 4). The tetrasporic plant,

¹ Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 9.

² The form reported from as far south as the Barbadoes by Mlle. Vickers (86) is believed by Dr. Farlow not to be identical with *G. Bornetiana*.

like the sexual individuals, sometimes produces reproductive organs when consisting of but 10–20 cells and having a height of less than half a centimetre.

One plant was seen in which a few antheridial branches occurred, while the majority of the filaments bore numerous procarps and cystocarps. In another case, most of the branches produced antheridia, but a considerable number bore at the nodes rings of cells resembling in all particulars tetraspore-mother-cells, with the involucre rays characteristic of the tetrasporic sorus. These tetraspore-like structures are described in detail on p. 671.

Griffithsia Bornetiana becomes conspicuous at Wood's Hole in the first half of July, and grows rapidly until it reaches its maximum development about the first week in August. Towards the middle of August the plants of all ages cease to produce new branches, and slowly become disorganized, losing their rich pink colour, and becoming easily detached from the substratum. At this season great quantities are washed up on exposed points like Nobska Point at Wood's Hole. After being torn loose from their fastenings, the plants float about in the water for days or even weeks, continuing to produce spores which were shown by experiment to be capable of germination. At this season, the tetrasporic plants frequently show a very robust habit, forming spherical masses upwards of 15 centimetres in diameter.

The spores develop quite rapidly in the open. Bits of cotton cloth, tied to piles near mature plants, showed in two weeks' time sexual plants with ripe antheridia and carpospores, and tetrasporic plants with mature spores. The largest of these plants showed eight orders of branching, and consisted of as many as 500 cells.

A noteworthy fact about the occurrence of the various forms is that tetrasporic plants are always more abundant, as well as on an average larger than sexual plants. During the first two weeks of August, 1907, more than 500 plants were collected, care being taken to collect every plant seen, and not to select the larger specimens. On one occasion 352 individuals were brought into the laboratory and sorted carefully. Of these 321 were found to be tetrasporic, 15 cystocarpic, and 16 antheridial. At another time more than 200 plants showed about the same relative proportions. In other words, there is on an average an equal number of antheridial and cystocarpic plants, and for each sexual plant about ten tetrasporic ones. An exact count was not kept of plants collected earlier in the season, but there seems to be no doubt that tetrasporic plants greatly predominate in number at all seasons.

The same relations are shown quite strikingly by *Champia parvula* and *Chondria tenuissima*, at Wood's Hole, and will probably be found to obtain in many other red algae. Similar numerical preponderance of tetrasporic

plants has been noted in *Laurencia* by Phillips (61), in *Polysiphonia* at Naples by Oltmanns (59, 1, p. 650), and in *Corallina* by Solms-Laubach (74). Professor Farlow states that among the red algae, 'tetrasporic plants are a good deal more common than sexual plants, and, in decidedly the majority of species which I have examined, I have had to look through a mass of tetrasporic plants before coming to any bearing sexual organs. In the great majority of Florideae the chances are decidedly in favour of finding tetraspores rather than sexual organs.'¹

METHODS.

Of various fixing fluids employed, the weak chrom-acetic-osmic acid mixture was found to be best for cytological details (Yamanouchi, 92, p. 425). The time of fixation varied from one to ten hours. Paraffin sections 3 and 5 μ thick were used almost exclusively for the finer details of cell structure; grosser anatomical features were found to be best made out from mounts *in toto*. The most successful stain employed was Heidenhain's iron alum haematoxylin (2 hours in the alum solution, 4 hours in the stain), followed by eosin in clove oil, as recommended by Miss Fraser (31).

Difficulty was experienced in obtaining material showing abundant nuclear figures. Plants brought into the laboratory and fixed at all hours after having been kept in running water showed almost no mitoses. Finally favourable material was obtained by fixing 'in the field' at eleven or twelve o'clock at night.

VEGETATIVE CHARACTERS.

The thallus forms a hemispherical tuft, and is composed of much-branched filaments, which are made up of large swollen cells placed end to end in series. The filaments radiate from a common point of attachment, the holdfast. In a plant of average size, from the base to the apex of a single filament, exclusive of the branches, there are twenty to thirty cells; in large specimens the number of cells in a single filament may be twice as great.

The cells differ greatly in shape and size in different parts of the filaments (Fig. 4). Toward the base of the plant they are approximately cylindrical below and much swollen toward the upper end. The cells nearer the tip of the filament become shorter and relatively thicker, of an obovate shape, and of a deeper colour. Those cells of the female plant which bear the older cystocarps become very much swollen toward their upper ends. In the male plants the terminal cells bearing the antheridia are almost globose. The following table gives a general idea of the sizes of the various cells in a filament composed of twenty cells.

¹ From a personal letter.

No. of cell.	Tetrasporic and cystocarpic plants.			Antheridial plants.		
Apex.	max. diam.	min. diam.	length.	max. diam.	min. diam.	length.
1	.060 mm.		.060 mm.	.65 mm.		.65 mm.
2	.130		.190	.50	.25 mm.	1.25
3	.250		.400	.40	.25	1.25
4	.320		.600	.40	.20	1.25
5	.320	.160 mm.	1.000	.45	.20	1.50
6	.400	.200	1.15	.45	.20	2.00
7	.400	.200	1.30	.45	.20	2.00
8	.400	.200	1.60	.50	.18	2.25
9	.450	.160	2.00	.55	.16	2.50
10	.520	.150	2.50	.60	.15	3.00
15	.580	.160	3.20	.60	.15	3.00
20	.640	.200	2.50	.65	.20	2.50

The cell-wall responds to the usual tests for cellulose. After the death of the cell, the wall swells greatly in aqueous fluids. When so swollen, it shows a plainly lamellate structure (Fig. 5), similar to that described for *Bornetia* by Correns (19).

The cytoplasm forms a thin layer over the inner face of the free portion of the cell-wall, averaging $.6\mu$ in thickness in the older cells. On the cross-wall it forms a thickened circular pad; adjoining pads are in communication through the intercellular pores. The cytoplasmic pad over the upper cross-wall averages in the larger cells 10μ in thickness in the centre, becoming thinner toward the edges. On the lower cross-wall the pad is usually thinner, averaging about 3μ in thickness. The pads are about evenly divided into a granular layer adjoining the sap-vacuole, and a denser homogeneous layer next the cross-wall (Fig. 6). Nuclei are quite abundant in the granular layer but are not of usual occurrence in the homogeneous portion of the pad. Spherical bodies of various sizes, probably of a proteid nature, occur commonly in both layers of the cytoplasm over the cross-walls.

In *Griffithsia barbata*, Berthold (7) found the cytoplasm divided into a clear outer layer and a granular inner layer, the latter containing the nuclei and the chromatophores. This seems to be the usual arrangement of the protoplasmic elements in the coenocytes of algae. In *Griffithsia Bornetiana* the cytoplasm becomes plainly differentiated into two layers only where it reaches a considerable thickness, as in the thickened pads mentioned above, and in very young cells in which the sap-vacuole is still small.

Intercellular connexions are conspicuous in living as in stained specimens by reason of the peculiar plugs which close the otherwise open pit between the cells. *Griffithsia* is an unusually favourable form in which to observe the intercellular connexions because of their large size. Evidence presented below is believed to be strongly in favour of the view, doubted by many workers, that even the older cells are actually in physical and organic connexion through the large open pores in the cross-walls.

A typical intercellular connexion is shown in Fig. 6. The pore in the cross-walls is closed on each side by a disk, which is the 'stopper,' or 'plug' of Archer (3). This disk is in direct contact with the thickened pad of cytoplasm lying on the cross-wall. Connecting the disks is a broad strand of thin clear cytoplasm, or, in some cases, several smaller strands (Fig. 8). In several instances, bits of the proteid substance normally present in the pad have been found in the cytoplasmic strand which connects neighbouring disks, apparently having been fixed in transit from one cell to another (Fig. 6). The middle lamella mentioned later as being formed in some cases in cell-divisions has not been demonstrated in the older intercellular connexions.

The size of the pore varies with the size of the cells which it connects. The average diameter of the disks, which is the same as that of the pores, is about $11\ \mu$ in the large cells at some distance from the apex.

In living and in unstained fixed material the disks are refractive colourless bodies. They stain heavily with nuclear dyes, particularly with Heidenhain's haematoxylin. Cytoplasmic stains, such as eosin, colour them much less intensely. They are soluble in Javelle water, as was pointed out by Kienitz-Gerloff (46). This fact, coupled with the fact that the disks are continuous on both sides with unaltered cytoplasm, gives support to the view, first expressed by Schmitz (70) that they are protoplasmic in nature.

The results obtained by various workers on intercellular connexions in the red algae are conflicting. Archer (3) described in *Ballia* pits which were at first open and later became closed by a plug, or 'stopper.' Schmitz (70) came to the conclusion that the pit is closed by a delicate membrane, which is pierced by many or several protoplasmic strands. Hick (42) thought he had demonstrated a simple protoplasmic strand passing through the open pit. Moore (55) found that a pit-closing membrane is pierced by one or several protoplasmic strands. Wille (88) described and figured a sort of sieve tube in *Cystoclonium*. Harvey-Gibson (39) found that in *Polysiphonia fastigiata* an actual protoplasmic connexion is present only in young stages and that later a plug closes the pore-canal. However, he mentions that from the edges of the plug fibrillar thickenings connect the neighbouring protoplasts. Kohl (49) regarded the matter of protoplasmic continuity in the Florideae as still unsettled. Kienitz-Gerloff (46) found that the pit is closed by a delicate membrane, and reached the conclusion that an unbroken connexion cannot be said with certainty to exist in the form studied (*Polysiphonia*).

The number of nuclei in a single vegetative cell is always large. Since the nuclei are approximately equidistant in each cell below the apex, it is evident that the number in a cell varies directly with the size of the cell. Estimates made from several preparations show that the large cells near the base of the plant contain, on an average, 3,000-4,000 nuclei. As the

cells become smaller toward the apex of the filament, the number of nuclei becomes correspondingly less. A subterminal cell of average size contains about 100 nuclei; an exceptionally large subterminal cell may contain as many as 500 nuclei. In the newly formed terminal cell the number is much less, varying from 12 or 15 to 50, or even 75. The terminal cells, however, like the other vegetative cells, are always multinucleate.

The occurrence of multinucleate cells is rather general in the older portions of the thallus of other Florideae, while the terminal cell is usually uninucleate (Davis, 22; Oltmanns, 59, ii, p. 89). Schmitz, who first called attention to this fact (67), showed also that in the different species of a single genus the number of nuclei in the cells varies greatly. For example, in the genus *Callithamnion*, all the cells in the thallus of *C. plumula* are uninucleate; in *C. corymbosum* the older cells are multinucleate; in *C. Borreri* even the youngest cells have two or more nuclei. Obviously, then, the number of nuclei in the cell is no index of relationship in the red algae.

The nuclei of *Griffithsia Bornetiana* are pretty uniformly distributed through the cytoplasm. While the distance separating them varies somewhat with the age and condition of the cell, usually it is 25–30 μ . Not infrequently several nuclei, with the cytoplasm immediately surrounding them, form small clumps which project into the central vacuole (Fig. 7). In the cytoplasmic pad on the cross-wall 10–15 nuclei usually form a ring around the intercellular pore (Fig. 8).

The size of the nuclei varies considerably with the age of the cells, as has been shown by Berthold to be the case in the coenocytes of *Codium* (6). In the young cells the resting nucleus is, on an average, about 4 μ in diameter just before nuclear division and less than half that just after mitosis. In the older cells the average diameter of the nucleus is 2–3 μ . In the young sporelings the nuclei are very small, measuring 1–2 μ in diameter.

The resting nucleus is nearly spherical or somewhat flattened against the cell-wall. It shows a large, densely staining chromatin-nucleolus in the centre. The size of the nucleolus varies from one-fifth to two-thirds the diameter of the nucleus. It is smallest at the time of complete rest of the nucleus, and grows larger as the time for mitosis draws near. Around the periphery of the nucleus a faint linin network is visible. This is connected with the nucleolus by faint radiating strands (Fig. 10). Immediately enveloping each nucleus is a zone of cytoplasm, which appears denser than the cytoplasm elsewhere, and which is probably to be considered of kineplasmic nature. The thickness of this zone is quite variable. It often becomes about one-third the diameter of the nucleus.

Nuclear division occurs regularly by mitosis, being found most frequently in the terminal cell. It occurs also commonly in the subterminal cell, less

commonly in the third cell from the apex, and rather infrequently in cells older than this.

The divisions of the nuclei of a single cell near the apex are almost, though not quite, simultaneous (Fig. 9). In general, the nuclei near the apex of the cell are at a slightly more advanced stage of division than those near the base. For instance, the nuclei near the apex may show stages of anaphase, or even of telophase, while those in the middle region of the cell are at metaphase, when the nuclei near the base have reached only the condition of prophase (Fig. 9). When there is an accumulation of protoplasm in the apex of the terminal cell preparatory to cell-division, the nuclei in this protoplasmic mass may divide considerably before the nuclei of the lower part of the cell. In the older cells the nuclei do not show the same simultaneity of division. Here small groups of nuclei may undergo mitosis while the majority of nuclei are in the resting condition. In the younger cells, however, when one nucleus divides, all divide, though not exactly synchronously.

In this connexion, it is interesting to note the behaviour of the nuclei in the multinucleate cells of other plants. In the sexual organs of various Phycomycetes, the numerous nuclei divide at the same time, as in the oögonium of *Saprolegnia* (Davis, 24), in the oögonia and antheridia of *Pythium* (Miyake, 54), *Albugo* (Stevens, 76), and *Peronospora* (Wager, 87). Simultaneous nuclear division is reported also in the plasmodia of *Fuligo* (Harper, 38), in *Plasmodiophora* (Nawaschin, 56), in the 'ascus' of *Hemiasci* (Juel, 44, Popta, 62), in the ascus of *Ascomycetes* (Harper, 37), in the basidium of *Basidiomycetes* (Maire, 53), and in the binucleate cells of Uredineae (Sappin-Trouffy, 66). Among algae, approximately simultaneous nuclear division is known in the germinating zygotes of desmids (Klebahn, 47), in the young colonies of *Volvox* (Overton, 60), in *Sphaeroplea* (Klebahn, 48), in *Hydrodictyon* (Timberlake, 80), in the antheridia of *Fucus* (Guignard, 36). In the vegetative cells of *Cladophora*, Strasburger found that nuclear division is not simultaneous, though he reports that several stages of mitosis are to be found in a cell at the same time, which seems to indicate that the stimulus to division affects more than one nucleus at a time. Among the Archegoniates, simultaneous nuclear division is figured by Miss Lyon for *Selaginella* (52), and seems to be the rule in the developing endosperm and in the early divisions in the fertilized egg of Gymnosperms (Coulter and Chamberlain, 20, pp. 20, 31, 41, 83, 98); in the free cell formation of the endosperm of many Angiosperms (Coulter and Chamberlain, 21, pp. 165-6, 172), and in the developing embryo sac (ibid., p. 87). In certain Leguminosae, Guignard (35) reports simultaneous nuclear division in the cells of the suspensor. The second mitosis in the gonotokonts of Archegoniates is simultaneous in the two nuclei.

Schmitz, in his studies on the nuclei of Siphonocladaceae (67, 68) does

not discuss the question of simultaneity of nuclear division, but leaves the reader to infer that the mitoses in a cell do not occur at the same time. The same is true of Berthold's work on *Codium* (6), and of Fairchild's account of *Valonia* (27).

Approximate simultaneity of nuclear division may be said to be a very general phenomenon in multinucleate plant cells.

The small size of the nuclei renders *Griffithsia* a rather unfavourable object for the study of the details of mitosis. The following account is based on observation of the nuclei in vegetative cells of the tetrasporic plants.

The nuclei are throughout their history very poor in linin. The chromatin of the resting nucleus is not, therefore, distributed on a linin reticulum, but is contained in a centrally placed, homogeneous nucleolus, or karyosome (Fig. 10). It seems possible that a small amount of chromatin is distributed on the peripheral linin network, but the bulk of it is certainly in the nucleolus. As the nucleus prepares for mitosis, it increases somewhat in size, becoming about $4.5\ \mu$ in diameter; the nucleolus also enlarges. Chromatin from the nucleolus, in the form of rather large granules, passes out to the periphery of the nucleus along faint linin strands (Figs. 14, 15), very much as was described by Wolfe in *Nemalion* (90). At the same time, the nucleolus becomes differentiated into faintly and darkly staining areas, the latter probably representing chromatin. The chromatin continues to pass out of the nucleolus until the whole chromatin content is distributed through the nuclear cavity in the form of granules, some of which are connected with one another by linin threads (Figs. 11, 12, 13, 14, 15, 16). The number of these granules seems in every case examined to be more than twice the number of chromosomes, and in some instances the granules become much more numerous. The granules now approach the centre of the nucleus, at the same time becoming fewer in number, probably by the fusion of separate granules (Fig. 17). As they move toward the centre, they become arranged roughly in a flat plate, though all the granules do not lie in exactly one plane (Fig. 18). While this is going on, a faint spindle is formed, apparently by the rearrangement of the linin threads (Figs. 18, 19). The spindle fibres are connected with small, darkly staining kinoplasmic caps, which lie on the nuclear membrane at opposite poles of the nucleus (Fig. 19).

At metaphase the spindle is seen to be short and broad and more or less truncated at the ends (Fig. 21). The nucleus is flattened at right angles to the axis of the spindle, so that it is broader than long. The chromosomes are closely packed on the equatorial plate, which is nearly as broad as the nuclear cavity. The nuclear membrane is intact, so that the whole spindle is intranuclear.

In addition to being closely packed, the chromosomes do not lie in

exactly the same plane, and it has been difficult to count them with certainty. Between the chromosomes lies a darkly staining substance that renders counting still more uncertain. Numerous estimates, made from polar views of the equatorial plates, vary from 11 to 14. The normal number of chromosomes in the nucleus of the vegetative cell of the tetrasporic plant seems pretty certainly to be 14 (Fig. 20).

The nuclear cavity is largest at the time of prophase, measuring as much as 5.5μ in diameter. At metaphase it is considerably smaller, averaging 5.5μ broad by 3μ long. A similar decrease in the content of the nuclear cavity has been noted by Yamanouchi in *Polysiphonia* (93).

At metaphase the group of chromosomes splits into two, which withdraw toward the opposite poles of the spindle (Figs. 22–23). In anaphase the daughter-chromosomes of each group are seen to be arranged somewhat in the shape of a watch crystal, with the concave surface toward the pole of the spindle (Fig. 23), as was figured in certain nuclear divisions in *Nemalion* by Wolfe (90). The two groups of chromosomes are connected by a few spindle fibres.

As the daughter-groups of chromosomes approach the kinoplasmic caps, the outlines of the individual chromosomes become lost in a dense mass of chromatin, which is to give rise to the nucleolus of the daughter-nucleus (Fig. 24). At telophase the mass of chromatin is in immediate proximity with the kinoplasmic cap. In the meantime the nuclear membrane, which becomes fainter during the course of mitosis, disappears, the original nuclear cavity becoming filled with cytoplasm, only a few faint striae remaining of the spindle (Fig. 25). The mass of chromatin resulting from each group of daughter-chromosomes becomes surrounded by a clear area, which is bounded by a faint nuclear membrane (Fig. 26). The kinoplasmic cap grows around the daughter-nucleus, whose organization is now complete.

The axes of the mitotic figures seem to bear no relation to the axis of the cell nor to the position of the cell-wall (Fig. 9). When the axis of the spindle is at right angles to the cell-wall, however, the daughter-nuclei shift their position at telophase so that a line connecting them is parallel to the cell-wall.

In the vegetative cells of the sexual individuals the behaviour of the nuclei in mitosis is in general similar to that in tetrasporic individuals. The number of chromatin granules which pass out from the nucleolus and become distributed through the nuclear cavity, while variable, is always much less than in the nuclei of the tetrasporic plant (Pl. L, Figs. 27, 28). The size of the nucleus at prophase is about the same in the two cases. At the time of metaphase, however, the cavity of the nucleus of the sexual plant is somewhat smaller than that of the tetrasporic plant. The number of chromosomes on the equatorial plate in the sexual plant is about seven,

though here, too, the counting is made difficult by the presence of a darkly staining substance between the chromosomes (Figs. 29, 30, 31).

Mitoses of the type described above have been observed in vegetative cells of various ages, in the hair-cells of the procarp and cystocarp, in the primary tetrasporic cells, in the stalk-cells of the tetrasporangia, in the involucre cells of the tetraspore-sorus, in the sporelings from tetraspores, and in the sporelings from carpospores.

No undoubted cases of amitosis have been observed. An appearance suggesting amitosis has been noted in the stalk-cells of the tetrasporangium and in the cells of the sporogenous lobes, and may possibly occur in the vegetative cells; but the small size of the nuclei renders exact observation on this point very difficult. It may be said with certainty, however, that the usual mode of nuclear division is by mitosis.

It may be well to compare at this point the behaviour of the nuclei in division with those of *Polysiphonia* (Yamanouchi) and *Nemalion* (Wolfe), the two other red algae which have been carefully studied from the cytological standpoint.

	<i>Resting nucleus.</i>	<i>Origin of Chromosomes.</i>	<i>No. of chroms.</i>	<i>Poles of spindle.</i>	<i>Nucleolus.</i>
Nemalion	Chromatin in central nucleolus	Chromatin passes to periphery	8 (16)	centrosomes	karyosome
Polysiphonia	Chromatin in peripheral network	Derived from chromatin network	20 (40)	centrosphere like bodies	plasmosome
Griffithsia	Chromatin in central nucleolus	Chromatin passes into nuclear cavity	7 (14)	kinoplasmic caps	karyosome

The chromatophores are numerous, small, oval or round, flattened bodies of rosy pink colour lying in the granular cytoplasm next the cell-wall. They vary considerably in size; on an average, each is about 3.5μ long, 2.5μ broad, and 1.2μ thick. Usually the outline of the chromatophore is smooth (Fig. 33), but occasionally it is toothed, as is true in other species of *Griffithsia*. In the younger cells the chromatophores are crowded together without definite arrangement. In the older cells they often occur in curved rows, which are arranged in the form of an irregular network (Fig. 32), as was described for other species of *Griffithsia* by Berthold (7), and for many genera of the Siphonocladaceae by Schmitz (67).

The number of chromatophores in a cell is very large. In an older cell of average size, about 400,000 were estimated to be present.

The chromatophores in the protoplasmic pads lying on the cross-walls are much fewer in number and smaller than in other portions of the cytoplasm.

Chromatophores apparently are absent from some lateral cells when first cut off; nor have they been seen in the young procarps, in the hair-cells, in the stalk-cells of the tetrasporangia, or in the young tetrasporangia. While no leucoplasts have been demonstrated in these cells, it is possible that they are present.

In dividing, the chromatophores simply pull apart. They first elongate and the pigment collects in each end; then they assume approximately a dumb-bell shape; and finally either separate completely, or more usually remain connected by a fine strand, as though the division were not quite complete (Fig. 33).

Starch is normally present in the vegetative cells, as has been found to be true of Florideae generally by Bütschli (15), Bruns (14), Kolkwitz (50), and others. It occurs as very small granules in circular groups, or as larger granules lying in the cytoplasm between the chromatophores. Each starch grain is rounded or oval, usually with a dark centre; no signs of lamination have been observed. Starch is especially abundant in sporelings, and in the cells of the attaching organ.

Besides the starch grains, there are normally present in the cytoplasm rounded masses of various sizes of what seems to be proteid material. These spheres usually occur in small groups, each group being surrounded by a clear area. The groups seem to be specially abundant in the cells at the time of nuclear division, and often simulate nuclei (Fig. 9). Spheres of what seems to be the same material are usually present in the pads of protoplasm lying on the cross-walls, and small bits have been observed lying in the cytoplasmic strands connecting neighbouring cells (Fig. 6).

Cell-division in *Griffithsia* is remarkable for the disparity in the size of the daughter-cells. It was first described by Wright (91), whose account was supplemented by the observations of Berthold (7).

In the vegetative cell, division occurs (1) by the cutting off of daughter-cells from the terminal cell of the filament, (2) by the cutting off of small dome-shaped segments from the upper borders of cells below the apex. The first type of division simply increases the length of the filament, the second results in the formation of a new branch.

There appear to be two methods of cell-division. The first occurs most commonly in the larger cells, and is always preceded by an accumulation of cytoplasm, nuclei, and to a less extent of chromatophores, which forms a dense, more or less homogeneous mass in the terminal portion of the apical cell (Fig. 34). A thin dome-shaped membrane is now laid down, with its convexity towards the apex, cutting a solid accumulation of protoplasm from the tip of the cell (Fig. 35). This membrane is formed simultaneously over its whole extent. There is no trace of cleavage in connexion with its formation, the protoplasm being in contact with it on each side. The nuclei appear to have nothing to do with its formation,

nor is its formation visibly associated in any way with nuclear division. The membrane is, however, never formed until there is an accumulation of nuclei and cytoplasm in the tip of the cell. The young cell, at first a solid cap over the tip of the apical cell, grows rapidly, soon acquiring a central vacuole (Fig. 36), and forming a typical vegetative cell. During the growth of the young cell, the cross-partition loses its convex appearance and becomes flattened. It becomes overlaid on each side by a cellulose wall, which does not cover the membrane completely (Fig. 37). There is left in the centre a circular area or pit, which is noticeable because of the early development of the cytoplasmic plugs described on page 642.

This is a very unusual method of cell-division in coenocytes (Davis, 25, pp. 452-3). So far as I know, it has been described in detail in no other form, though a somewhat similar process appears to take place in the large vesicles of *Valonia* (Schmitz, 67).

The details of the division which gives rise to a lateral branch are very similar to those of the division of the apical cell. The daughter-segments of the subterminal cells are formed on the side of the upper border of the cell, usually four or five cells from the apex. There is first a solid accumulation of protoplasm, then the adjoining cell-wall bulges outward, and a dome-shaped membrane cuts the outer part of the protoplasmic mass from the inner, precisely as in the division of the apical cell. The young segment pushes out and becomes cylindrical, a vacuole early appearing in its middle, and forms the apical cell of a new branch (Fig. 38). The branch thus initiated grows for a time more rapidly than the main filament, until it about equals it in size. Thus an apparent, or false, dichotomy results. True dichotomy appears never to occur, as in no case has a branch been found to divide longitudinally. Frequently more than one branch is laid down at a node, so that trichotomy results, and in the larger cells near the base of the plant, four branches have been observed to proceed from the summit of the same cell.

A second method of cell-division occurs commonly in the apical cells of the smaller branches, and sometimes in the division of the larger cells below the apex. A ring of cellulose projects inward from the cell-wall a short distance from the apex, very much as was described for *Cladophora* by Strasburger (77) (Figs. 39, 40, 41). The ring grows inward, but not so as to cut off the new cell completely. An open circular pore is left in the centre, across which the protoplasmic plugs are soon formed in the usual way. No pit-closing membrane is formed between cells separated in this manner.

The second method of division, which is the usual one in coenocytes, differs from the first in that the daughter-cell is from the beginning much more nearly equal in size to the cell from which it is cut than is the case in the first method of division.

Wherever the second method of cell-division occurs, the partition is in one plane, never arched. In all the cases examined in which division occurred by the ingrowth of a cellulose ring, the partition cut into the central vacuole, so as to cut off a segment containing part of the vacuole of the mother-cell; in the first method of division, however, the segment cut off is at first solid. The relation of the cleavage plane to the vacuole seems to determine the method of cell-division; in the division of the vegetative cells, where the cleavage plane occurs so as to cut into the solid protoplasmic accumulation in the apex of the cell, division takes place by the first method. Where the plane of division is sufficiently removed from the apex to allow the partition to cut into the central vacuole, division is by the second method.

As mentioned above, the nuclei appear to take no part in cell-division. This seems to be the rule in coenocytic cells (Strasburger, 77), though Wille has noted an apparent exception in *Acrosiphonia* (89).

The number of nuclei in the smaller daughter-cell just after its formation is various. The average number is between 12 and 20, but in some cases, and always following the second method of cell-division, the number is considerably greater. The cell next below the apex may show 30-250 nuclei.

Branched hairs are frequently borne on the upper borders of the younger cells. There are usually six or seven of these around each node on which they occur.

Their mode of origin is very similar to that of the tetrasporic filaments to be described later. Small papillae arise nearly simultaneously around the upper border of a cell near the cross-partition, each papilla containing a single nucleus and dense, homogeneous cytoplasm. The papillae are cut off from the protoplast by an arched membrane, similar to that formed in the division of some of the vegetative cells. The nucleus now divides, and one of the daughter-nuclei wanders into a bud from the papilla, the bud, with its nucleus, now becoming cut off. A second, a third, and sometimes a fourth bud are formed and cut off like the first. Each of these daughter-cells behaves in the same way, cutting off three or more buds, and in this way a thrice compound hair is formed (Fig. 42). Each of the terminal cells divides into two. The basal cell of a hair becomes multinucleate, as do the cells of the first order of branching; the cells of the second order of branching remain uninucleate. The basal cell is connected with the cell on which it is borne by an intercellular connexion of the same type as that which occurs between neighbouring vegetative cells.

After they are fully formed, the hairs elongate greatly and become hyaline. Each cell takes part in the elongation. A vacuole is formed in the cytoplasm which increases in size as the cells elongate. A very thin layer of cytoplasm lies between this vacuole and the cell-walls; in the outer

ends of the long cells are seen accumulations of cytoplasm, in which most of the nuclei lie.

The fully formed hairs may remain a considerable time before elongating. Elongation occurs in all the hairs of a single node at the same time, and seems to take place rather suddenly. The total length of a hair of average size before elongation is about 40μ , and after elongation about 350μ . Such great increase in size in a short time seems to be rendered possible by the fact that the cells of a hair do not secrete a cellulose wall until after elongation has taken place.

After elongating, the hairs remain for a while on the plant, but finally the connexion between the basal cells and the vegetative cell breaks, and the hairs fall off as a whole. Not infrequently a second crop of hairs is formed before the first crop falls off, so that there appear to be 'two sets of hair-like organs' (Farlow, 29, p. 132). By the time the second set is formed, the first set is carried by the growth of the vegetative cell to some distance from the cross-partition between the vegetative cells, and the second set of hairs is always formed between the cross-partition and the first set (Figs. 42, 43).

Individuals vary greatly in the number of hairs produced. In some specimens, hairs are found on almost every node in the younger portion of the plant. Again, one may look over a great many shoots before encountering a single set of hairs. What external conditions are favourable to the production of hairs in *Griffithsia* is not known.

In the material examined by Miss Smith (73), hairs occurred on the female plant only on nodes bearing cystocarps. Such a restricted distribution is not general. Any of the young vegetative cells seems to be capable of producing hairs, and while hairs occur usually in the vicinity of reproductive organs, there seems to be no necessary connexion between the two.

The function of the hairs is quite unknown. They undoubtedly increase markedly the surface exposed to the water, and inasmuch as they occur especially abundantly in the neighbourhood of the reproductive organs, where the processes of metabolism may be assumed to be most active, and are usually absent on the sterile portions of the plant, it seems likely that they perform the functions of absorption and respiration, as is believed by Rosenvinge (65) to be the functions of similar organs in the Rhodomelaceae.

Rhizoids are frequently formed from the older vegetative cells. Protoplasm accumulates at a spot on the lower half, or near the middle, or even at times on the upper half of the cell, and pushes out as a hollow tube with a plug of protoplasm at its tip (Fig. 44). In the cytoplasm of a rhizoid the chromatophores are rather few in number, and the nuclei are smaller than usual in vegetative cells. The average diameter of a rhizoid is about 80μ , the length 2 mm. or more. The rhizoid secretes a rather thick cellulose wall. The longer rhizoids become divided into two or three long cylindrical

cells by the centripetal growth of a cellulose ring such as occurs in the division of certain vegetative cells.

The rhizoids so formed attach themselves to any neighbouring object, curving around it in the manner of a tendril (Fig. 45). In this way the plant is more securely anchored than it would be by a holdfast alone. Further, rhizoids frequently become entangled among neighbouring filaments of the same plant, thus binding the lower parts of the filaments more closely together and rendering them less easily torn apart (Fig. 46). This is especially true of the antheridial plants, in which the rhizoids are very richly developed.

After the rhizoids become attached, new shoots may arise from them in the same way that lateral branches arise from the vegetative cells.

Tendrils have been known in the red algae since Agardh (1) first described them in *Hypnea*, *Mychodea*, *Rhabdonia*, and other genera. Setchell (71) described tendrils in *Laurencia* and *Cystoclonium*, and stated that they may also serve for vegetative propagation. Nordhausen (57) described tendrils in *Hypnea*, *Spyridia*, and *Nitophyllum* and showed that new plants may arise from root-tendrils in *Hypnea*.

A species of regeneration occurs in the filament when, as is often the case, one of the old cells perishes. Continuity of the filament is re-established in the following way: An outgrowth from the cell next above pushes through the intercellular pore, and grows down into the cavity of the dead cell. The outgrowth is a tube, similar in appearance and mode of formation to a rhizoid. The cavity of the outgrowth is perfectly continuous with the cavity of the cell from which it originates. A similar tube grows up more slowly from the cell below, and the two meet near the centre of the old cell cavity (Figs. 47, 48). They fuse at their tips (Fig. 49) to form a continuous hollow cylinder; the cylinder increases in size and comes to replace the dead cell exactly. The usual intercellular connexion is formed at the junction of the new cell with each of the two old cells which contributed to its formation. A similar process of regeneration was described for *Griffithsia Corallina* by Janczewski (43), with this difference, however, that in *G. Corallina* only the cell above the dead cell plays a part in the formation of the new cell. Tobler (83) has shown that a similar process takes place in other species of *Griffithsia* and in *Bornetia*.

This process leads at times to the production of a cell of very peculiar appearance. When the cell next below two branches perishes, the lowest member of each branch puts out a tube (Fig. 47) which meets the tube from the cell below. The three fuse at the point of contact and a Y-shaped cell results, which is a product of the fusion of three distinct cells (Fig. 50).

Griffithsia may be anchored to the substratum either by a special attaching disk, or more usually by a tangled mass of rhizoids. An attaching disk has been noted in plants growing on *Zostera* and smooth rocks, but

when, as is often the case, *Griffithsia* is attached to other algae of cylindrical habit the disk is replaced by a tangled mass of rhizoids, which are short, thick-walled, and filled with starch. Inspection of a number of specimens shows all stages of transition from a mass of rhizoids to a well-developed attaching disk. The disk may be said to be formed of rhizoids in contact laterally. The development of the attaching organ is described on page 676.

The attaching disk, when present, is formed of a single layer of heavy-walled cells, bright pink in colour owing to the presence of numerous chromatophores, densely filled with protoplasm, and packed with large starch grains (Figs. 51, 52). From it new shoots may arise.

Judging from analogy with other forms (see Oltmanns, 59, i, p. 648; and ii, p. 212) we may assume that the plant winters over by means of the attaching disks or the mass of rhizoids at its base. In the spring these give rise to the plants which reach perfection in the summer. The evidence for this is rather negative: 1. The first plants found in 1907 possessed the attaching disk already well developed, whereas we know that in the sporelings the basal disk is developed quite slowly. 2. From the time *Griffithsia* was first found in July development was extremely rapid, and this seems to point to the conclusion that the plants drew on some reserve food-supply.

SEXUAL REPRODUCTION.

The antheridia are distributed as caps over the upper ends of the somewhat globose terminal cells of the male plants (Fig. 2). They are formed as the terminal cells of short, much branched antheridial filaments. On a cell of average size there were found to be about 500 of these filaments, each of which produce about 50–75 antheridia, a total of 25,000–37,500 antheridia for each fertile cell of the male plant. The number of antheridia on a single antheridial filament, as well as the number of filaments produced on a single cell, varies greatly.

The mode of origin of the antheridial filaments is as follows: While the terminal cell of the male plant is still small and not much swollen (measuring on an average 0.2 mm. long and 0.15 mm. broad at this stage) about 100–200 protuberances arise simultaneously on its apical surface (Fig. 53). Each protuberance is at first hemispherical. There is in each a single nucleus, surrounded by dense, clear cytoplasm, which is in free communication with that of the mother-cell (Fig. 54). Their formation is not connected with nuclear division, but takes place while the nuclei are in the resting condition. The withdrawal of so many nuclei from the upper portion of the parent-cell leaves this region almost free of nuclei. As growth proceeds, however, nuclei wander up from the basal region, and become again evenly distributed in the cytoplasm.

Each primary protuberance is soon cut off from the mother-cell by a delicate partition, which is laid down by the protoplasm in the same way as

in the first-mentioned cell-division described on page 649 (Pl. LI, Fig. 55). When its formation is complete, the primary protuberance divides several times vertically. A lateral cytoplasmic process is formed, then the nucleus divides by mitosis (Figs. 56, 57). One daughter-nucleus remains in the body of the primary protuberance, the other passes into the cytoplasmic process. The two protoplasts now become separated by a constriction of the *Hautschicht*, whose ingrowth appears to be aided by the formation of a vacuole at the point of constriction (Fig. 57). This process of division continues until there are about 200–500 secondary protuberances on the apical portion of the terminal cell.

The protuberances appear not to develop cellulose cell-walls of their own, but lie in the swollen wall of the mother-cell (Figs. 58, 59).

Each of these secondary protuberances gives rise to a branched antheridial filament. The single nucleus in each divides by mitosis, and a partition is formed separating the two nuclei, and cutting the protuberances into an upper and a lower cell. The lower or basal cell buds off other cells above, each uninucleate, to the number of five or six (Fig. 59). These, along with the upper of the two cells first formed, in turn bud off groups of uninucleate cells, which become the spermatia directly (Fig. 60). Thus the antheridial filament is a twice compound structure like a small bush, the terminal twigs of which become the spermatia.

The basal cell of the antheridial filament is somewhat cubical in shape and may contain ultimately several nuclei. The cells of the first and second order of branching are uninucleate and are remarkable for their shape, each resembling a pear with a very long stem. These cells early become filled with a large vacuole, the cytoplasm forming a very thin film next the *hautschicht*, and the nucleus lying in the apical portion. None of the cells of the antheridial filament appear to form a cellulose wall. The whole filament is covered by the swollen wall of the mother-cell (Fig. 59). When the spermatia are mature, they simply break loose from the cells on which they are borne, and float freely out into the water (Fig. 63). As they become free, the long neck which attached them to the cell next below becomes drawn into the body of the spermatium, which assumes an oval shape.

The mature spermatium is about 3μ long and 2μ in diameter. Its bulk is occupied by a large vacuole, which is bounded by a thin film of cytoplasm. The single nucleus lies in the end which pointed away from the antheridial filament. No chromatophores have been discovered in any of the antheridial cells. The living spermatium is quite clear and somewhat refractive.

It seems of interest to note the fact that the living spermatia appear not to be extruded unless a slight pressure is exerted on the cells of the thallus. If branches of an antheridial plant are transferred carefully from

sea-water to a slide and left undisturbed, few antheridia become extruded. However, the pressure of a cover-glass, or even a mere touch with a needle, causes the extrusion of hundreds of antheridia from each large antheridial cap. This, coupled with the fact that the tufts of *Griffithsia* are feeding-grounds for several species of minute crustacea, especially of a species of *Caprella*, seems to lend probability to the suggestion that the antheridia of the red algae are sometimes translocated through the agency of animals.

No evidence was secured as to whether the antheridial filaments produce successive crops of spermatia. It is certain, however, that after a time the antheridial cap ceases to produce spermatia, and the antheridial filaments become disorganized and break away, leaving the globose terminal cell of the thallus free of any antheridial cells. When this occurs, it is usual for two or more side-branches to arise from the subterminal cell and to begin to produce antheridia when three or four cells long (Fig. 64).

The globose terminal cell which has produced one crop of spermatia frequently produces one or more new branches from its summit, so that this cell becomes again a functional apical cell (Figs. 64, 65).

The procarps occur laterally on the nodes near the tips of the filaments of the female plants (Fig. 3). They are produced successively, so that on a fertile branch a procarp is formed on nearly every node. Their origin and development has been described in detail by Miss Smith (73), whose account supplements that of Farlow (29) and Spalding (75).

The procarps are formed from the small terminal vegetative cells. When a procarp is to be initiated, the terminal cell, instead of dividing in the way usual in terminal vegetative cells, becomes pushed to one side by a lateral branch of the subterminal cell (Fig. 66, 67) which becomes the main axis of the filament. The terminal cell which is to give rise to the procarp contains several nuclei; it divides into two cells in such a way that one cell lies partly over the other. The plane of division is oblique, the inner edge of the partition being somewhat lower than the outer (Fig. 67). The lower of the two cells so formed is the basal cell of the procarp. The upper cell divides again by a transverse wall to form the central cell of the procarp and the first peripheral cell (Fig. 68). The first peripheral cell is cut off from the central cell on the axial side. A second and third peripheral cell becomes cut off from the upper border of the central cell, with no discernible regularity of position (Figs. 69, 70). The fourth peripheral cell mentioned by Farlow has not been seen, and must occur only occasionally. Of the peripheral cells only one has any further part in the production of carpospores. Often, though not always, the peripheral cells cut off terminally a small sterile cell (Fig. 71).

Up to this point the number of nuclei in each of the cells of the procarp varies from 4 or 5 to 10 or 12 or more; there appear to be always

more than one. As the procarp develops, the number of nuclei increases by mitosis, until in the cells of the mature procarp the nuclei become quite numerous. The average numbers are about as follows: basal cell, 50; central cell, 45; peripheral cells, 8–30; each sterile cell, 4.

The cytoplasm in the cells of the young procarp is homogenous and rather dense. A vacuole of considerable size occupies the centre of the basal cell and of the central cell. No chromatophores or leucoplasts have been seen in the cells of the procarp, though chromatophores are developed in the cells of the cystocarp.

It sometimes happens that after the first three cells of the procarp are formed the procarpic branch becomes metamorphosed into a vegetative shoot of the usual type, but distinguishable from other vegetative shoots by the fact that the cell at its base remains broad and flat, retaining the appearance of a basal cell of a procarp (Fig. 72). In such cases the first peripheral cell, which is really a terminal cell, functions as an apical cell of a vegetative branch.

From the second or third peripheral cell the carpogenic branch is formed laterally. The peripheral cell from which the carpogenic branch is produced is the 'supporting cell' of Miss Smith, and is equivalent to the 'auxiliary cell' of Hassencamp (40). In this account I shall adopt the term auxiliary cell.¹ From it a small uninucleate cell is produced laterally, which is the basal cell of the carpogenic branch. This cuts off a terminal cell, which in turn divides (Fig. 71). The upper of the three cells so formed divides again, thus forming a carpogenic branch of four cells (Fig. 73). The carpogenic branch is bent at right angles in such a way that the terminal cell, from which the carpogonium and trichogyne are formed, is usually in contact with the auxiliary cell. Each cell of the carpogenic branch is at first uninucleate. From the free border of the terminal cell the trichogyne is produced as a club-shaped projection (Figs. 73, 74). Whether a division of the nucleus of the carpogonium accompanies the formation of the trichogyne, as has been found to be the case in *Batrachospermum* (Davis, 22), *Nemalion* (Wolfe, 90), and *Polysiphonia* (Yamanouchi, 93), has not been determined. The trichogyne increases in length, becoming 35–40 μ long, and becomes quite slender, with a diameter of about 1 μ . In the mature trichogyne several granules, which stain like chromatin, are to be observed (Fig. 78a).

The mature trichogyne is straight or somewhat curved and sometimes, though not always, slightly swollen at the free end.

Before and during the formation of the carpogenic branch, noteworthy changes take place in the auxiliary cell. The cytoplasm becomes denser and the nucleus nearest the centre of the cell increases very greatly in size.

¹ The terminology used in the present account of the procarp and cystocarp is essentially that employed by Miss Smith, with the exception noted.

Before the differentiation of the auxiliary cell, the nuclei may average 1.5μ in diameter; when the carpogenic branch is fully formed, the central nucleus of the auxiliary cell may reach a diameter of 6.5μ (Fig. 76). A similar change may take place in one of the nuclei of the other peripheral cells.

The structure of the mature procarp is as follows (Figs. 73, 75): The broad, flat basal cell bears on its upper border the central cell, which is also broad and rather flat. The central cell bears usually three peripheral cells which may or may not cut off sterile cells at their tips. One of the peripheral cells bears laterally the carpogenic branch, which consists of a basal cell, two intermediate cells, and the carpogonium with its trichogyne. The intermediate cells and the carpogonium are disposed in a straight line, which lies at right angles to a line passing through the basal and the auxiliary cells.

All the cells of the procarp are multinucleate except those of the carpogenic branch. Of these the terminal cell and the two intermediate cells are uninucleate, and the basal cell of the carpogenic branch is usually binucleate. The connexions between the cells of the procarp appear to be similar in general to the connexions between neighbouring vegetative cells.

Mention has been made above of the hairs which usually occur in the vicinity of procarps.

The small size of the trichogyne and of the carpogonium renders *Griffithsia Bornetiana* a rather unfavourable object for the study of the details of fertilization, but it has been possible to make out the essential facts. A spermatium becomes attached to the trichogyne near its tip (Figs. 75, 77, 78). The spermatium is either applied directly to the surface of the trichogyne, or there may be a short tube connecting the two. Whether the nucleus of the spermatium divides at this stage, as is the case in *Nemalion* (Wolfe, 90) was not determined, though such an appearance as is presented in Fig. 78 suggests that division may occur. A sufficient number of stages was not obtained to enable me to speak with certainty on the subject but the stages that were obtained seem to render it highly probable that the nucleus from the spermatium passes down the trichogyne, enters the carpogonium, and there fuses with the nucleus of the carpogonium (Figs. 77, 79).

Immediately after fertilization the trichogyne becomes much twisted and falls off, leaving a short stump on the carpogonium (Fig. 80). Since the fusion nucleus stains very heavily, the details of its structure were not made out. However, because of this very capacity for taking up dyes, it is easily distinguished from the other nuclei of the procarp.

Very soon after fertilization, the carpogenic branch begins to be withered, and the fusion nucleus is seen to be present in the auxiliary cell

(Fig. 81). The actual passage of the fusion nucleus into the auxiliary cell was not observed. In no case has a fusion nucleus been seen in any of the cells of the carpogenic branch other than the carpogonium, and it seems unlikely that it passes into any of these other cells. The position of the carpogonium in contact with the auxiliary cell renders it possible that the two become connected by resorption of the walls at the point of contact, and that the fusion nucleus passes directly into the auxiliary cell, as was suggested by Schmitz for other species of *Griffithsia*, and has been demonstrated in *Thuretella* and *Chylocladia* by Hassencamp (40). In *Polysiphonia violacea* the communication between the carpogonium and the auxiliary cell is transient (Yamanouchi, 93), so that it might well be difficult to demonstrate in such a form as *Griffithsia*.

The cells of the carpogenic branch after fertilization and the passage of the fusion nucleus into the auxiliary cell usually degenerate simultaneously, and often the whole carpogenic branch breaks away from its attachment to the auxiliary cell, and lies free among the cells of the procarp (Fig. 82). In one case the lower cells of the carpogenic branch were seen to have withered before the passage of the fusion into the auxiliary cell, which lends support to the view that the fusion nucleus passes directly into the auxiliary cell and not through the cells below the carpogonium. Miss Smith's account (73, p. 41) of the withering of the carpogenic branch was not corroborated in the present study. She states that the carpogonium first becomes disorganized, 'the adjacent cell at the same time apparently increases in size, but it also soon loses its contents, and in some cases appears to become disorganized, while the two lower cells take a deeper stain than before'. As stated above, the carpogenic branch usually withers as a whole, and not cell by cell.

At the time of the passage of the fusion nucleus into the auxiliary cell there is in the centre of the latter a very large clear nucleus. This is one of the nuclei originally present in the auxiliary cell. Besides this, two or three small nuclei are frequently seen in the peripheral portion of the cell (Fig. 81); these are the remaining nuclei present at the time of the organization of the auxiliary cell. They seem to disappear during the course of the further development of the auxiliary cell.

The fusion nucleus in the auxiliary cell is of very characteristic appearance. It differs from the usual type of nucleus in possessing two chromatin-nucleoli instead of one. It would seem as if the chromatin from the male and the female parent does not fuse completely, and that the nucleoli of different origin remain distinct for some time after nuclear fusion. The behaviour of the chromosomes in the early divisions of the fusion nucleus was not observed, though it would be of considerable interest to know whether two distinct groups of chromosomes are formed at this stage.

The fusion nucleus divides once in the auxiliary cell, and the two nuclei come to lie in the opposite ends of the now somewhat elongated cell (Fig. 83). Between them lies the greatly enlarged central nucleus originally present. Each of the nuclei resulting from the division of the fusion nucleus usually shows the characteristic double nucleolus. The auxiliary cell now divides, one daughter-cell containing the enlarged central nucleus and a single fusion nucleus, and the other containing only a fusion nucleus (Fig. 84). The latter may be called the placental cell; from it the sporogenous lobes usually arise. Fig. 86 shows clearly that sporogenous lobes may also be formed from the auxiliary cell after the placental cell has been formed; the nuclei entering these lobes are derived from the fusion nucleus. Very similar behaviour has been observed by Hassencamp (40) in the auxiliary cells of *Thuretella* and *Chylocladia*.

During these changes, the large nucleus of the auxiliary cell continues to increase in size. It becomes almost empty of contents, the nuclear outline becoming less and less distinct, and finally the nucleus disappears in the cytoplasm.

Changes also take place in the other elements of the young cystocarp. The central cell, which at first contains a large central vacuole, becomes filled with homogeneous cytoplasm and with numerous nuclei formed by the multiplication of those originally present (Fig. 81). From the sides of the basal cell of the procarp soon after fertilization several small cells are cut off successively. These in turn divide, and the outer cell becomes an involucre ray (Fig. 85). Three to seven involucre rays are formed; they are of various sizes and ages, and curve up over the cystocarp so as to cover it almost completely, except at the top. The structure of the involucre rays offers nothing especially remarkable. They are distended sacs, pale pink in colour owing to the presence of a small number of chromatophores. A thin layer of protoplasm bounds a very large vacuole. The nuclei may become quite numerous, 87 having been counted in a ray of average size. The rays do not form a definite pericarp, as they are not united at the sides. None are produced between the cystocarp and the vegetative cell which bears it.

The placental cell formed at the division of the auxiliary cell increases in size and in number of nuclei, all of which are the product of the division of the fusion nucleus. Small protuberances are formed on its free border, each of which contains a single nucleus (Figs. 85, 86). Each protuberance is cut off from the mother-cell by an arched membrane, and the cells so formed give rise by repeated division to the sporogenous cells from which the carpospores are formed.

While this is taking place, there is a general fusion of cells in the centre of the cystocarp. The following cells take part in this fusion: The placental cell, the auxiliary cell, the central cell, and sometimes the

peripheral cells. The result of the fusion is the production of a very large, irregularly shaped placenta, on the upper surface of which the sporogenous lobes are formed (Figs. 85, 87).

The placenta contains nuclei from three sources: (1) the original nuclei of the peripheral and auxiliary cells, which appear to take no part in spore formation; (2) the numerous nuclei of the central cell, which lie in the base of the placenta, a region from which no sporogenous lobes are formed; and (3) the numerous nuclei resulting from the division of the fusion nucleus. These last lie in the upper region of the placenta, where the sporogenous lobes are being formed, and appear to be the only nuclei to enter these. A similar placenta, with numerous nuclei of diverse origin, has been described in *Chylocladia*, by Hassencamp (40).

At least in some cases, the nuclei from the central cell appear to become abnormal and break down. The chromatin forms a crescent-shaped mass applied to the nuclear membrane on one side (Fig. 88), the nuclei swell, their outlines become faint, and finally their contents mingle with the cytoplasm. Not all of the nuclei from the central cell degenerate, and it is often difficult to distinguish those which remain normal from the sporogenous nuclei, especially in the older cystocarps, except by their position in the cell.

The mode of division of the sporogenous lobes seems to vary considerably in different lobes. The series of figures from 89-93 (Pl. LI and LII) gives a fair idea of what usually takes place. Following the division of the nucleus of one of the protuberances mentioned as being formed on the free surface of the placental cell or on the upper part of the placenta, small curved segments are cut off from the outer surfaces of the protuberance in much the same way as a segment is cut off from the apical vegetative cell, with this difference, however, that the cells of the sporogenous lobes are usually uninucleate. In this way a compact tissue is formed, the cells of which round themselves off, each cell producing a carpospore. As the cells round off, the sporogenous lobe becomes converted into branched chains of oval cells. The links of a chain are free at the sides, but connected with each other above and below by a narrow strand of cytoplasm; midway between connected spores occur callus-like plugs similar to those lying in the pits between adjacent vegetative cells. From the time when they first round off, the spores increase greatly in size. When the spores are ready to be shed, their diameter is about 30-35 μ , their length 40-54 μ , the diameter of the nuclei 8.5-9 μ .

This is the usual history of a sporogenous lobe. In some cases a difference is presented because of the fact that the spore-mother-cells are rounded off at a very early age, so that the sporogenous lobe is not a compact tissue when young, but a group of rounded cells (Figs. 94, 95). There is some evidence that in this case the method of cell-division in the

sporogenous lobe is by constriction, somewhat after the manner of the second method of cell-division described on page 650. The final result is the same in the two cases, i. e., the production of branched chains of carpospores.

The sporogenous lobes of a single cystocarp are of various ages. Lobes with mature spores may be seen by the side of unicellular sporogenous lobes, and usually all stages of development may be seen in a single cystocarp (Fig. 95).

Each sporogenous lobe is covered with a gelatinous envelope, not easily seen until swollen with glycerine or a watery fluid. The individual spores seem to be without a cellulose wall, being enclosed only by the *hautschicht*.

As a rule, all the spores of a single lobe become mature at the same time. The links of the chains break at the point where the callus-like plugs are developed, the connecting strands are drawn into the body of the spores, and the spores slip out of the gelatinous envelope and float away into the water.

The number of spores produced in a cystocarp can hardly be estimated with certainty, because while the mature spores are being shed, new sporogenous lobes are being inaugurated. From a study of several cystocarps of average size, it was found that about 6 lobes are present at one time, with an average of about 40 spores in each lobe, giving a total of about 240 spores in a normal cystocarp. Undoubtedly the number of spores produced during the life of a cystocarp may often be greater than this.

When set free, the spores present much the same appearance as the tetraspores described on page 670. They are oval in shape. Around the periphery is a zone containing rather dense cytoplasm and numerous flattened chromatophores, which sometimes present their edges, but usually their flat surfaces, to the outside. In the centre is the large nucleus, enveloped in a zone of homogeneous cytoplasm. The nucleolus, which contains the chromatin, is usually in the form of 12–14 rounded bodies in the centre of the nuclear cavity. The linin is scanty in amount, being barely visible around the periphery of the nucleus. Between the nucleus and the peripheral zone of chromatophores, the cytoplasm is very coarsely vacuolar (Fig. 96).

In a few cases, spores have been noted which have germinated *in situ*, and which contain two nuclei.

Nuclear divisions in the cystocarp are of the usual type. In the divisions of the sporogenous nuclei, the number of chromosomes is about fourteen (Fig. 97).

Food material appears to be passed into the cystocarp from the vegetative cell on which it is borne. In the cytoplasmic pad occurs a great abundance of the spheres of food material mentioned on page 649, and it

seems probable that this material is passed up into the cystocarp from the cell below.

ASEXUAL REPRODUCTION (Tetraspores).

The tetraspores are formed in a ring around the upper border of any cell below the apex of the filament (Fig. 4). The ring of tetraspores appears to encircle the node, fitting snugly in the constriction between neighbouring vegetative cells. On the outside of the tetraspores a circle of involucrial rays grows up around the sorus. The number of these is variable; there are often only six or eight; sometimes there are as many as twenty. They appear rather late in the development of the sorus, in some cases the most advanced tetraspores having already matured before the involucrial rays are formed. The rays are expanded curved plates, connected at the base with the vegetative cell, and free laterally and terminally. Usually neighbouring rays are in contact at the sides, so that the circle of tetraspores is well screened from without. Each ray is a single cell similar in appearance and in structure to the outer cell of the involucrial ray of the cystocarp. Where the rays are in connexion with the vegetative cell, the plugs characteristic of the intercellular connexions elsewhere are formed.

The tetraspores are formed as follows:—Around the upper border of a young cell below the apex, protoplasm accumulates in small rounded masses, each containing a single nucleus (Fig. 98). The cell-wall near each protoplasmic accumulation becomes gelatinous, which allows the accumulations to protrude as small papillae (Fig. 99). Each of these papillae early becomes cut off from the mother-cell by a delicate dome-shaped membrane (Fig. 100). The cell so formed, with its nucleus, increases in size, and at the same time the membrane loses its convex form and becomes flattened (Fig. 101).

The formation of these primary tetrasporic cells seems to take place entirely independent of nuclear division.

On the upper border of each of these cells a finger-like outgrowth of cytoplasm is protruded (Fig. 102). The nucleus then divides by mitosis in the way described for vegetative nuclei of the tetrasporic plant (Fig. 103). One of the daughter-nuclei remains in the basal portion of the cell, the other passes into the cytoplasmic outgrowth, a membrane appearing between the nuclei (Fig. 104). Thus is formed a small two-celled branch, with a single nucleus in each cell (Fig. 105). The lower may be called the stalk-cell, while the upper is the tetrasporangium or tetraspore-mother-cell.

During the growth of this structure, the stalk-cell pushes out another cytoplasmic projection similar to the one first formed. The nucleus divides by mitosis (Fig. 106), one of the daughter-nuclei remaining in the stalk-cell, and the other passing into the projection, which becomes cut off like the first. Thus a second tetraspore-mother-cell is formed on the stalk-cell.

A third and sometimes a fourth mother-cell may be formed in the same way. The first mother-cell may be regarded as terminal, the other as lateral.

In rare cases, two nuclei occur in the primary tetrasporic cell from its inception, and in one case, at least, two nuclei have been noted in the very young tetraspore-mother-cell. This recalls the suggestion of Heydrich (41) of a possible sexual significance of the tetraspore. Examination of a large series of developing tetraspore-mother-cells convinces me, however, that there is here a purely accidental phenomenon, which has no place in the normal life-history, and which is not to be considered as analogous in any way to the sexual process.

The cells of the tetrasporic branch appear at first not to secrete cellulose walls of their own. The stalk-cell, with its tetraspore-mother-cells, remains surrounded by the gelatinized wall of the vegetative cell on which it is borne. This wall, much swollen, covers the tetrasporic branch completely (Fig. 107). It continues to swell, and by the time the spores are ready to be discharged it seems to dissolve largely or completely in the sea-water.

The stalk-cell increases in size, and its nucleus at the same time divides by successive mitoses until usually sixteen daughter-nuclei are finally produced. With the growth of the cell in size, the cytoplasm becomes less dense, and vacuoles appear in it. There may be a single large central vacuole or several smaller ones variously disposed. The connexion of the stalk-cell with the vegetative cell, and also with the tetraspore-mother-cells, is of the usual type. On the side toward the stalk-cell the cytoplasm of the mother-cell is produced into a rather narrow strand, which meets a similar strand from the stalk-cell at the point where the callus-like plugs are developed (Fig. 109).

It sometimes happens that the stalk-cell produces laterally a tubular process that curves up around the mother-cells and resembles in appearance an involucre ray (Fig. 110). This recalls the condition in *Griffithsia barbata* and other species, in which the tetraspores are borne laterally on short involucre ramuli.

The tetraspore-mother-cell increases in size, the nucleus showing corresponding enlargement. The cytoplasm begins to show numerous small vacuoles between the rather dense cytoplasm surrounding the nucleus and that lying in the periphery of the cell.

The behaviour of the nucleolus during the period of enlargement of the nucleus is interesting. As the nucleolus increases in mass, it fragments into several rounded bodies of various sizes (Figs. 107, 108). This process of fragmentation continues until from 12-14 rounded masses of chromatin of about the same size are formed (Fig. 111). These lie in a clump in the centre of the nucleus, staining very heavily with nuclear dyes.

At this stage the tetraspore-mother-cell may be considered to be mature. The length of a mature mother-cell is about $20\ \mu$, the width $15\ \mu$, and the diameter of the nucleus $7\ \mu$. Further changes in the mother-cell are in anticipation of division into tetraspores.

From this time the changes in the cytoplasm occur mainly in connexion with the vacuolar area. The vacuoles become larger and the whole vacuolar area presents a coarse spongy appearance. In the meshes are deposited numerous spheres of substance staining deeply with haematoxylin. There is reason to believe that these bodies are derived from the nucleus. As the time of nuclear division approaches, these granules become larger and fewer in number, so that it is possible, by noting their size and number, to predict in just what stage of mitosis the nucleus will be found. The granules seem to be analogous to the chromidial substance of Protozoa (see Goldschmidt, 33) and of some plants (see Tischler, 81).

The changes in the nucleus are profound. Most striking is the decrease in staining capacity of the nucleolar masses. These become irregular in form, and at the same time fuse with one another, so that their number is reduced by more than half (Fig. 112). At this stage they are in the form of thick, curved rods, in which light and darkly staining areas may be discovered. Often four dark areas may be detected in each rod, which suggests that this stage corresponds to the formation of tetrads in the oocytes and spermatocytes of many animals. Coincidentally with these changes, small granules are to be seen in the nucleus near its periphery, which seem to pass out into the cytoplasm (Figs. 112, 113), to form the granules already mentioned as occurring in the vacuolar area.

The stage just described is considered to be the period of synapsis. It is of long duration, it shows a condition which does not occur elsewhere in the life-history, and it immediately precedes the mitoses in which numerical reduction of the chromosomes takes place. It differs from the usual type of synapsis in that no spirem or synaptic thread is formed; but this is not to be wondered at inasmuch as nowhere in the life-history of *Griffithsia Bornetiana* is a spirem produced. Perhaps the worm-like nuclear masses are to be considered as replacing the usual spirem stage. Somewhat similar conditions in the nucleus of the zygote of *Spirogyra* are interpreted by Karsten (45, p. 6) to represent the period of synapsis in this form.

While the thick, irregular rods continue to lose their capacity for taking up stains, there appear scattered throughout the nuclear cavity, but mainly near the periphery, a number of small spherical or oval bodies (Figs. 114, 115). About fourteen of these bodies are usually present, though the number may vary within narrow limits. This is the stage of prophase, and the small bodies are the chromosomes. At this time traces of the achromatic substance of the nucleolus may be detected near the centre of the nuclear cavity.

Not all the nucleolus goes to form the chromosomes. As already mentioned, part of the nucleolar substance passes out of the nucleus and becomes deposited in the vacuolar cytoplasm, and part may remain in the nuclear cavity, where it forms irregular masses. The part remaining in the nucleus ultimately disappears in the cytoplasm after the nuclear membrane is dissolved.

The details of the organization of the spindle are made out with difficulty. During prophase, kinoplasmic caps are formed at the poles of the nucleus by differentiation of cytoplasm at these points. In most cases, at the centre of each kinoplasmic mass is a darkly staining body (Fig. 116), probably comparable to the 'centrosphere-like-structures' of *Polysiphonia* (Yamanouchi, 93). In some cases these are large and prominent; in others they could not be demonstrated at all. They are certainly not permanent structures; they seem rather to be the expressions of some temporary kinoplasmic activity. To them the spindle fibres are attached. The spindle is entirely intranuclear, and is probably differentiated from materials within the nuclear cavity, as no evidence has been seen to indicate that the fibres grow in from without, as is the case in *Spirogyra* (Berghs, 4). The spindle is truncate at the poles and slightly broader at the equatorial plate (Figs. 116, 117). The chromosomes, which lay scattered in the nuclear cavity before the formation of the spindle, now move in toward the centre of the nucleus (Fig. 118); here they become arranged on the equatorial plate. Some preparations (Fig. 119) seem to indicate that during this process they become associated in pairs, which soon separate; but on this point it is impossible for me to speak with certainty at present. The number of chromosomes in the equatorial plate is approximately fourteen. They are small rounded bodies, not lying exactly in the same plane (Fig. 120).

The axis of the spindle seems to bear no constant relation to the axis of the cell. It is more usual, however, to find the long axis of the spindle coincident with the long axis of the mother-cell. The outline of the nucleus at metaphase is nearly circular, or more often, slightly elongated in the direction of the axis of the spindle (Fig. 116).

At anaphase the chromosomes separate into two groups, probably of seven each (Fig. 121). As the groups of chromosomes approach the poles of the spindle, the nuclear membrane fades away, and the cavity of the nucleus is obliterated by the cytoplasm. In some cases, however, this does not happen; the nuclear membrane persists throughout mitosis. During anaphase, it elongates and then pulls apart in the middle (Fig. 122). Whether this diversity in the behaviour of the nuclear membrane is in any way connected with certain irregularities of development to be described later, is not obvious.

As each group of chromosomes approaches the pole of the spindle, the individual chromosomes unite to form a densely staining spherical mass,

which becomes the nucleolus of the daughter-nucleus. When the original nuclear membrane persists, the organization of the daughter nuclei is complete on the separation of the two halves of the nucleus, by which time no trace of the spindle is seen. When, as is more usual, the nuclear membrane disappears toward telophase, the mass of chromatin in the immediate vicinity of the kinoplasmic cap becomes surrounded by a new nuclear membrane, around which the kinoplasm becomes distributed.

In any event, two daughter-nuclei are formed, which lie at some distance from each other. Each has a large, spherical, uniformly staining nucleolus in the centre, and frequently with two or three smaller bodies of chromatin in the nuclear cavity (Fig. 123). The daughter-nuclei are considerably smaller than the nucleus of the mother-cell. Each is about $5\ \mu$ long by $6\ \mu$ broad, though the size varies. No trace of any partition has been observed between the daughter-nuclei, which lie quite freely in the cytoplasm.

The daughter-nuclei do not remain long in the resting condition. In each the nucleolus disappears, and seven rounded chromosomes, probably derived from the nucleolus, appear in the nuclear cavity (Fig. 124). At the same time the nucleus elongates further, and there is to be seen a kinoplasmic cap at each end. A spindle is organized as before, and the seven chromosomes arrange themselves in an equatorial plate. The division of the two nuclei is synchronous, their axes of division lying at right angles to each other (Fig. 125). At anaphase, two groups of seven chromosomes pass to the poles of each spindle, the nuclear membranes disappearing (Fig. 126). At telophase the chromosomes of each group which are in close proximity to the kinoplasmic cap, fuse to form the nucleolus of the daughter-nucleus. A new nuclear membrane is formed around each mass of chromatin and the kinoplasm again becomes distributed around the nucleus.

The four nuclei thus formed lie very near the periphery of the mother-cell, and equidistant from one another (Fig. 107). Each is a definitive tetraspore nucleus. Their arrangement in the cell is determined by the fact that one nucleus always lies at the point from which the cytoplasmic strand passes to meet the stalk-cell. The structure of the nucleolus at this stage is somewhat different from that of the preceding stages. The chromatin mass is usually plainly lobulated. Outside and near this is to be seen a much smaller, regularly spherical body, whose history I have been unable to trace. Probably it is of the same nature as the nucleolus, since, when the nucleolus fragments, as it does a little later, the smaller body is indistinguishable from the other chromatin masses.

An appearance frequently seen at this stage lends support to the view that food material is passed up from below into the tetrasporangium (see Yamanouchi, 93, p. 424). The nucleus which lies near the strand of cytoplasm connecting the tetrasporangium with the stalk-cell is seen to be

surrounded by a mass of food material, which is probably derived from the stalk-cell. The other nuclei at the same time lie in clear cytoplasm in which little stored food is visible (Fig. 127).

During the progress of these changes in the nuclear content of the tetrasporangium, the deeply staining granules in the cytoplasm disappear, so that by the end of the first mitosis they are no longer visible. At the same time, the large vacuoles in the cytoplasm give place to smaller, more regular ones.

Cleavage of the cytoplasm begins always when the four nuclei begin to move toward the centre of the tetrasporangium, which happens soon after their formation. The *hautschicht* folds inwards along planes which, if continued to the centre of the cell, would cut the protoplast into four equal parts, presenting the familiar tripartite arrangement (Fig. 128). However, the partitions are produced inwards only about two-thirds of the distance to the centre of the cell (Pl. LIII, Fig. 129). The central portion of the tetrasporangium is occupied by the four definitive spore nuclei with their envelopes of kinoplasm which are in contact with one another, so that a rather definite nucleo-kinoplasmic mass is formed. In the very centre of the tetrasporangium lies the portion of the undifferentiated cytoplasm enclosed by the nucleo-kinoplasmic mass (Fig. 130).

The nuclei at this stage are either spherical or somewhat biscuit-shaped, the inner surface being less convex than the outer. The nucleolus has by this time fragmented into 12-14 granules, similar in appearance to those in the nucleolus of the tetraspore-mother-cell before synapsis.

At this point of development 5-10 per cent. of the tetrasporangia begin to show degenerative changes and do not develop further. The outer surface of the tetrasporangium becomes wrinkled, and the nuclei become very much flattened, almost wafer-like. The entire contents of the protoplast stain very heavily, owing to the presence in the cytoplasm and in the nuclei of numerous dark granules. These degenerating tetrasporangia are easily distinguishable, even in the living condition, by reason of their dark, opaque appearance, and some are to be found in every tetrasporangial sorus. What causes lead to their degeneration I have not been able to determine.

In the normal tetrasporangia, the cleavage partitions, which represent folds of the *hautschicht*, but which appear in section as a single line except near the periphery (Fig. 129), split so as to reveal clearly their double nature. At the same time the cytoplasm, which lay in close contact with the partitions, separates along the line of the partitions so that the cleavage furrows become wide as well as deep (Fig. 130). Even at this stage, however, they extend no further in than to the edge of the nucleo-kinoplasmic mass. Coincident with these changes in the partitions, the nuclei which were flattened become again approximately spherical.

Vacuoles develop in the cytoplasm in the centre of the nucleokinoplasmic mass. At the same time, small chromatophores begin to appear in the cytoplasm along the outer border of the tetrasporangium. These increase in size, and a few extend along the partitions into the body of the protoplast. In this condition the tetrasporangium remains for a long time, increasing in size and in vacuolization of the cytoplasm. The significance of this incomplete separation of the spores probably lies in the fact that food material seems to pass up through the basal cell. If the spores were completely separated before maturity, only one of the four would be in communication with the stalk cell, the source of supplies. However, inasmuch as the chromatophores at this stage are well developed, it seems probable that the tetrasporangium is capable of elaborating at least part of its food material for itself.

Berthold (7) seems to have been the first to point out this incomplete separation of the tetraspores of red algae after the division of the nucleus of the mother-cell, though Schmitz (69) had given an account of two successive nuclear divisions in the tetraspore-mother-cell.

The tetrasporic branches are from their inception surrounded by the swollen wall of the vegetative cell on which they are borne. A portion of this wall is carried out by the developing tetrasporic cells. As the cells develop, the portion of the wall surrounding them swells greatly and appears to become gelatinized, ceasing to respond to the tests for cellulose.

The tetrasporangium, with the incompletely separated spores, increases markedly in size. The nuclei also enlarge and show abundant chromatin, in the form of the 12-14 masses already mentioned. Each mass is differentiated into lightly and darkly staining areas. Not infrequently the number of these masses is greater than this, as many as twenty having been counted in some cases; and sometimes the number is considerably less than twelve. This variability in the number of chromatin masses in the resting nucleus serves to show that they do not have the same constancy in numbers that the chromosomes show, and therefore are not to be relied on as an index of the condition of the nucleus, whether haploid or diploid.

As the tetrasporangium enlarges, the cytoplasm becomes more coarsely vacuolate, and the vacuoles in the central protoplasmic mass become conspicuous. The partitions now grow in until they meet in the centre of the tetrasporangium, their ingrowth being apparently aided by the position of the large central vacuoles already mentioned (Fig. 131). The spores are now completely separated, with the nuclei in the inner corners. The nuclei wander toward the centre of the spores, the chromatophores at the same time migrating so as to line the entire periphery, and the spores round off, becoming oval in shape (Fig. 132). The lowest spore, up to this time attached to the stalk-cell by a slender thread of cytoplasm, breaks away at the point of attachment, and the strand is withdrawn into the body of the

spore. The gelatinized cell-wall, now very much swollen, appears to dissolve in the sea-water, and the four spores are set free, almost immediately becoming spherical. Like the carpospores, they are heavier than sea-water, and slowly sink if left undisturbed.

The mature tetraspore resembles the carpospore in appearance. It is approximately spherical. In the centre the large nucleus is conspicuous, with its chromatin segregated into 12–20 small masses. Immediately around the nucleus is a zone of rather dense cytoplasm; outside this the cytoplasm is coarsely vacuolar. In the peripheral cytoplasm is a single layer of chromatophores, outside which is the limiting membrane of the spore. No cellulose cell-wall is visible.

The average size of the tetrasporic structures is shown in the following table :—

	<i>Cell.</i>	<i>Nucleus.</i>
Mature mother cell	15–20 μ	7 μ
Mother cell at synopsis	20 $\times$ 24 μ	8 μ
Four nuclei peripheral	20 $\times$ 24 μ	4–6 μ
Four nuclei central	25–30 μ	4–6 μ
Partitions separate	40–50 μ	8 μ

The tetrasporic structures in a single sorus are of very various ages. While the first-formed tetraspores are developing, new tetraspore-mother-cells are being formed nearer the cross-walls between the vegetative cells, the older tetraspores being carried away from the cross partition by the growth and the stretching of the wall of the vegetative cell. A longitudinal section of a sorus shows primary tetrasporic cells being formed very near the point of junction of the vegetative cells; outside these are the older tetraspore-mother-cells; farther out mature spores are to be seen; while farthest from the centre occur the involucre rays (Fig. 133).

The number of tetraspores produced in a single sorus is quite large. The average number in well developed sori was found to be about 300.

The process of nuclear division in the tetraspore-mother-cell of *Griffithsia* offers many striking points of difference from the same stages in the life-history of *Polysiphonia*; it resembles much more nearly similar stages in *Corallina* (Davis, 23). As these three forms are the only members of the Rhodophyceae in which the behaviour of the tetraspore-mother-cells has been carefully studied from a cytological standpoint, it may be well to summarize here some of the points of resemblance and difference :—

	<i>Griffithsia.</i>	<i>Corallina.</i>	<i>Polysiphonia.</i>
Resting nucleus : chromatin	In large central granules	In scattered granules of varying number	In reticulum
Resting nucleus : nucleolus	Karyosome with some plasmosome substance	Plasmosome	Plasmosome
Synapsis : chromosomes	Broad irregular bands, free at ends	?	Double spirem
Origin of chromosomes	From central nucleolar bodies	From scattered chromatin granules	Segmentation of spirem
Fate of nucleolus	Achromatic portion disappears at prophase or persists till telophase	Disappears at prophase	Disappears after prophase
Daughter-nuclei	Assume resting condition	Assume resting condition	Not organized
Second mitosis	In daughter-nuclei		Inside membrane of mother-nucleus
Nuclear membrane	Disappears or pulls apart after metaphase of first division	Disappears before metaphase of first division	Persists through both divisions

This comparison serves to emphasize one point particularly. At a critical stage in the life-history of rather closely related members (*Polysiphonia* and *Griffithsia*) of a highly specialized group, the cytological phenomena are of a most varied nature. During the period of synapsis, and up to the time of the formation of the chromosomes, the cytological events in *Polysiphonia* are more like those in *Lilium* than those in *Griffithsia* or *Corallina*. The behaviour of the nucleus in the formation of the tetraspores in *Griffithsia* is much more similar to that in *Corallina* than to that in *Polysiphonia*, a more nearly related genus. The bearing of these facts is obvious; cytological phenomena cannot be considered trustworthy guides to relationships.

TETRASPORE-LIKE STRUCTURES ON SEXUAL PLANTS.

As stated above, one individual has been found which produced normal antheridia on the majority of its filaments, but which produced on a considerable number of filaments structures resembling the sori of tetraspores. As this case is of considerable theoretical interest at the present time, I shall now give some of the details of the structure of this plant.

The portion of the plant bearing antheridia was perfectly normal in appearance. The cells were of the usual size and shape, and bore antheridia of the normal type in abundance. The number of chromosomes appearing in mitoses in the antheridial filaments was found to be about seven (the reduced number, characteristic of the gametophyte). Mitosis has as yet

been seen in only a single nucleus of the portion of the plant bearing the tetraspore-like structures. In this case, in the dividing nucleus of the stalk-cell, the number of chromosomes was seen to be seven (see Fig. 135).

The branches bearing tetraspore-like structures are of the same type as those of the normal tetrasporic plant, but are composed of cells that are on an average a good deal smaller.

The early development of the tetraspore-like structures is similar in every detail to corresponding stages in the development of the normal tetraspores. Small uninucleate papillae are cut off from the upper border of the vegetative cells. Each divides to form a short two-celled branch, the lower cell representing the stalk-cell of the tetrasporangium, the upper corresponding to the tetraspore-mother-cell (Figs. 134, 135). The stalk-cell increases in size and becomes vacuolate, the mother-cell also becomes larger, but remains somewhat smaller than the normal tetraspore-mother-cell. The average diameter of the fully formed mature mother-cell is about $20-22\ \mu$ as against $24\ \mu$ for the mother-cell of the tetraspore. The nucleus in the two cases shows the same configuration, but remains smaller in the mother-cells borne on the sexual plant (Fig. 135). Nuclear material passes out into the cytoplasm, where it forms small darkly staining granules.

Involucral rays are formed in the ways characteristic of the tetraspore-sorus, usually as outgrowths from the vegetative cell outside the ring of spore-mother-cells, exceptionally as lateral outgrowths from the stalk-cell (Fig. 136).

The further development of the mother-cells on the sexual plant differs strikingly from that of the normal tetraspore-mother-cells. In the majority of cases the nucleus divides (whether by mitosis or amitosis I have not yet been able to determine), and cleavage begins at the periphery (Fig. 137). The cleavage furrows do not advance far into the body of the mother-cell. The surface of the cell begins to show irregular wrinkling, and degenerative changes set in similar to those described for certain tetraspore-mother-cells. The number of nuclei in cells in which cleavage furrows begin is usually 4-8, of which some are very much larger than the rest (Fig. 137).

One case deserves special mention. Sixteen nuclei lie scattered in the cell which shows no trace of the formation of cleavage furrows (Fig. 138). The whole cell presents the appearance of a germinating spore. It would seem that here the cell corresponding to the tetraspore-mother-cell behaves as a monospore, though whether such a cell ever produces a normal plant is uncertain.

The chromosome-history of the nuclei of the cells just described has not yet been determined.

VEGETATIVE MULTIPLICATION.

Griffithsia Bornetiana may reproduce itself vegetatively in two ways: first, by accidental isolation and subsequent growth of single cells or small pieces of a filament; second, by the production of new plants from tendrils.

The first method of propagation was described for *G. Corallina* by Janczewski (43) and mentioned as occurring in *G. Bornetiana* by Farlow (29). More recently Tobler (82, 83, 84) has called attention to the fact that *Griffithsia*, *Bornetia*, *Dasya*, *Polysiphonia*, and other forms may reproduce themselves under laboratory conditions by a process of fragmentation of the filaments and growth of the resulting portions into new plants. In *G. Bornetiana* this process takes place not rarely in nature. In such cases the isolated cell produces a rhizoid from its base and a new growing point from its apex (Figs. 139, 140). The rhizoid is formed normally in the manner already described. The apical cell is produced by the accumulation of protoplasm at the tip and subsequent unequal division of the parent cell in the usual manner.

Vegetative propagation by means of tendrils has been described on page 653.

GERMINATION OF SPORES.

The spores germinate readily in the laboratory. If a mature tetrasporic or cystocarpic plant be placed in sea-water over night, young sporelings up to the 3-celled stage will be found abundantly attached to the bottom and sides of the vessel the next morning. Many of the stages of germination here described were collected in the field under natural conditions, but the majority of the figures given, especially of the younger stages, were taken from material cultivated in the laboratory.

The similarity of the structure of the carpospores and the tetraspores has been noted above; the phenomena of germination are also practically the same in the two kinds of spores. On being released, the spores become spherical and settle slowly in the water. They appear to become attached to the surface of any solid body they touch, such as rocks, glass, other algae, and even such soft bodies as the gelatinous substance enclosing chains of diatoms.

During the progress of germination, soon after the spore becomes attached, there is formed around it a cellulose wall of the usual type, which becomes tolerably thick, especially around the basal region of the sporeling. At the same time, numerous starch-grains also become visible in the cytoplasm of the spore. Several hours after the spore is shed the nucleus divides by mitosis. During this time there is no noticeable change of shape in the spore.

Opportunity has not occurred for the examination of a large series of dividing nuclei in the sporelings, but in the cases examined the mitoses were of the type usual in vegetative nuclei. In the dividing nuclei of sporelings from tetraspores, about six or seven chromosomes appear on the equatorial plate (Fig. 143). In the sporelings from carpospores the number of chromosomes is always greater than this, but appears to be less than the number that might be expected (14). There is believed, however, to be sufficient evidence for regarding these nuclei as diploid in character.

The daughter-nuclei withdraw to opposite sides of the sporeling (Fig. 141) and divide again to form four nuclei, which in turn divide to form eight (Fig. 142), then sixteen (Fig. 143). The increase in the number of nuclei is not accompanied by a corresponding increase in the size of the spore; the size of the nuclei becomes less with each succeeding division. At about this time, the sporeling changes its shape, pushing out, at the point of attachment, a small rounded projection, which later becomes cut off as the basal cell. Immediately after this projection is formed the sporeling elongates, becoming about twice as long as broad, but without undergoing cell-division (Fig. 144).

As these changes take place the cytoplasm migrates more and more to the periphery. The central part of the sporeling is occupied by small regular vacuoles, whose exceedingly thin walls are roughly hexagonal in section (Fig. 141). A single large central vacuole such as is characteristic of the vegetative cells of the older plants is not usually formed until the sporeling reaches the 3-celled stage.

Cell-division occurs usually when about sixteen nuclei are present. A wall at right angles to the long axis of the sporeling cuts off a basal from an apical cell (Fig. 145). Shortly after this, without further elongation of the sporeling, the apical cell divides into two by a wall parallel with the first (Fig. 146). The details of the formation of these walls were not followed out. As the partitions have not been seen to assume the arched shape characteristic of the first type of cell-division at other points in the life-history (see p. 649), it may be inferred that they are formed by the ingrowth of a ring of cellulose (p. 650), such as is formed in the divisions of the cells of *Cladophora*. From the two divisions a small obovate 3-celled sporeling results, which consists of a smaller, somewhat pointed basal cell and two larger rounded cells towards the apex, the three cells lying in a row (Fig. 147). At this stage, the chromatophores and protoplasm are so closely packed in the peripheral portion of the cytoplasm that the sporeling appears dense and opaque. The pointed end of the basal cell is filled only with homogeneous protoplasm, starch-grains, and chromatophores being absent from this region. Intercellular connexions were not demonstrated in the small 3-celled sporelings, though they are apparent after the enlargement of the cells.

The description given above applies to the majority of sporelings examined. Many sporelings, however, show variations from the type described. For instance, it happens rather frequently that cell-division occurs after the formation of only four nuclei (Fig. 145) and before the sporeling assumes the elongated shape represented in Fig. 144.

The 3-celled stage is first attained in about twelve hours from the time the spore is shed. It persists, under laboratory conditions, for several days, during which time the three cells change greatly in size, shape, and appearance. The most striking changes occur from the second to the third day after the spores are shed. The cells increase greatly in size, and a large central vacuole is formed in each. The protoplasm and inclusions being spread over a larger area form a thin film, in which the chromatophores are no longer crowded, but are separated by considerable clear spaces; the sporeling therefore becomes lighter in colour and more transparent. The following comparison of sporelings of the second with those of the third day gives some idea of the great increase in the size of the cells.

	<i>Length in mm.</i>		<i>Diameter in mm.</i>	
	<i>Second day</i>	<i>Third day</i>	<i>Second day</i>	<i>Third day</i>
Apical cell	·037	·133	·067	·073
Middle cell	·037	·467	·067	·1
Basal cell	·047	·107	·04	·073
Total	·121	·707		

The number of nuclei in the cells, even after enlargement, is small. Several counts indicated that there are in the apical cell on an average 25–30 nuclei, in the middle cell 20–25, in the basal cell 5–10. These nuclei in the resting condition are very small, averaging, perhaps, 1 μ in diameter. In structure they resemble the nuclei of the older vegetative cells.

The changes in the apical and middle cells consist mainly of (1) a great increase in length, (2) slight increase in breadth, and (3) the distribution of the protoplasm and inclusions over a much larger area.

In the basal cell, besides an increase in size, the most striking changes are those of shape. These changes depend to a large degree on the substratum. In case the sporeling is attached to some soft body, such as another alga, the basal cell remains somewhat top-shaped, with the pointed end applied to, or in some cases wedged into the substratum (Figs. 148, 149, 152, 153). If, however, the sporeling is attached to a hard body, such as glass or stone, the basal cell becomes greatly elongated, in some cases coming to equal in length all the rest of the sporeling (Figs. 150, 151). When this occurs, the basal cell resembles strikingly a rhizoid of the older plant.

When the stage just described is reached there seems to come a natural pause in the life-cycle. In a state of nature a great many more sporelings are found in the 3-celled stage than in any other, indicating that this stage occupies a longer time in the course of development than any other. Under laboratory conditions the 3-celled stage is retained at least several days, and frequently development goes no further. The factor determining further development seems to be, in part at least, the character of the substratum. In the cultures examined it was found that on glass or on clean, though rough stones, the basal cell continued to elongate, though without further division of the apical cell, until the whole sporeling lost its natural colour and died. However, in case the elongating basal cell came in contact with some soft substance, it fastened itself immediately, and normal development proceeded. In a state of nature, young sporelings of *Griffithsia* have been most commonly found at Wood's Hole on *Champia parvula* and on *Lomentaria uncinata*, though they occur on other algae and on *Zostera marina*. Young plants were sometimes found on stones, the surface of which appeared clean, but proved, on careful examination, to bear other sporelings, to which the plantlets of *Griffithsia* were probably at first attached. There is no evidence of parasitism, however, in the early development of *Griffithsia*. Sporelings flourish on any soft substratum, such as bits of cotton cloth. From the observations noted above, it seems clear that *Griffithsia Bornetiana* needs some other substratum than the stones on which the mature plant is often found, to pass through the early stages of its existence.

When the sporeling is growing on some other alga, the basal cell may simply become attached to the surface by some adhesive at the surface of contact (Fig. 152), or may grow in between the cells of the algal substratum (Fig. 153), or may even twine about it in the manner of the rhizoidal tendrils.

Further development of the basal cell results in its division into cells of various sizes and irregular shape. Usually short tubular projections resembling rhizoids become cut off (Fig. 154) by the circular ingrowth of the cell-wall. Somewhat less frequently dome-shaped segments are formed on the sides of the basal cell (Fig. 155). In either case a multi-nucleate holdfast, or attaching disk is formed, all the cells of which are derived from the division of the basal cell (Fig. 156).

The apical cell cuts off daughter segments in the usual manner (Fig. 157). By the time the sporeling is four or five cells long, lateral branches appear on the upper borders of the cells below the apex (Fig. 158). The rapid growth of the lateral branches gives the characteristic false dichotomy to the young thallus. Branching is profuse near the base of the sporeling. Frequently five or six lateral branches are given off from each of the lower cells, so that the young plant is copiously branched. In this

event the cells bearing numerous branches become thick-walled and almost globose in shape.

It is interesting to note that when numbers of sporelings are found in immediate vicinity in nature, all are often at precisely the same stage of development. For instance, about fifteen sporelings were observed on a single branch of *Lomentaria*; all were at the stage of germination represented in Fig. 158.

Hairs are usually wanting in the young plants, nor are rhizoids developed except from the basal cell.

The phenomena of germination noted above agree in all essentials with the account of *Griffithsia Bornetiana* given by Miss Derick (26), and are in line with the phenomena reported in other species by Tobler (85).

It is of considerable interest that the coenocytic condition characteristic of the cells of the mature plant is attained in the sporeling before any sign of cell-division or differentiation. The recapitulation theory¹ has been shown in many cases to be applicable to other plants, e. g. in the formation of the megaspores of the Hydropteridineae (Strasburger, 79), in the forms of juvenile leaves (Berry, 5), in the post-embryonal stages of the Laminariaceae (Setchell, 72, and Griggs, 34), in the formation of the eggs of the Fucaceae (for the facts, see Oltmanns, 59, ii, pp. 47-8). If this theory is at all applicable to *Griffithsia*, we should expect some evidence of it at the times in the life-history when the plant returns to the unicellular condition. If there is virtue in the conclusions drawn from comparative morphology, the ancestors, and even the comparatively recent ancestors, of *Griffithsia* possessed uninucleate cells. The coenocytic habit was acquired late in the history of the race, and we should expect it, therefore, to appear late in the history of the individual, so that the cells of the early stages would be uninucleate; yet in the germinating spore of *Griffithsia* the first visible change is the attainment of what we must regard as the recently acquired coenocytic habit. In this respect, then, *Griffithsia* does not conform to the recapitulation theory.

DISCUSSION OF RESULTS.

From the cytological evidence brought forward in this paper it seems probable that there exists in *Griffithsia Bornetiana* an alternation of generations similar to that which has been suggested for *Polysiphonia violacea* (Yamanouchi, 93). The fusion nucleus, which contains fourteen chromosomes, with the co-operation of the cytoplasm of some of the cells of the procarp, produces the cystocarp, in which are formed carpospores;

¹ 'A highly organized plant, which begins its development with the simplest stages and gradually advances to a state of higher differentiation, repeats in its ontogeny its phylogenetic development.' Strasburger, Noll, Schenck, and Schimper: A Textbook of Botany, Second English edition, 1903, p. 49.

the nucleus of each of these contains fourteen chromosomes. The nuclei of the tetrasporic plant contain each fourteen chromosomes; and it therefore seems reasonable to assume that the tetrasporic plants arise from carpospores. In the first division of the nucleus of the tetraspore-mother-cell the number of chromosomes is reduced one-half, so that seven chromosomes enter the nucleus of each tetraspore. It seems probable that on germinating the tetraspore gives rise to an individual whose general morphological relations and vegetative structure are similar to those of the plant producing tetraspores, with two significant exceptions, (1) the nuclei show at mitosis seven chromosomes instead of fourteen, and (2) the individual bears sexual organs instead of asexual spores. In other words, in *Griffithsia* a sexual plant is probably succeeded by an asexual plant of similar morphological relations.

The proof of this hypothesis must rest on actual cultural experiments, and it is much to be desired that such experiments be carried out.

Since Strasburger (78) showed that in the Archegoniates the double number of chromosomes is characteristic of the sporophyte and the single number of the gametophyte, the main facts have been confirmed in so many forms that many botanists have come to consider that the chromosome number alone is a trustworthy guide for the identification of the two generations: that plants showing the diploid condition of the nucleus necessarily belong to the sporophyte, and that where the haploid condition of the nucleus obtains, the gametophyte is necessarily involved. Botanists have come to speak of the sporophyte as the '2 x -generation' (Lotsy, 51), and of the gametophyte as the ' x -generation'; and undoubtedly within the Archegoniate series such a conception is very useful. However, even in Archegoniates, where the rule is so generally applicable, recent work tends to show that the diploid condition of the nucleus is not necessary for the differentiation of the sporophyte (Yamanouchi, 94), nor is the haploid condition necessary for the differentiation of the gametophyte (Farmer and Digby, 30).

In thallophytes, the evidence at hand indicates great diversity in the point at which the numerical reduction of chromosomes takes place. Even in the single group of Rhodophyceae the point of reduction occurs at different places in the life-history of different species. When one comes, therefore, to regard the chromosome number as the sole test for the delimitation of sporophyte and gametophyte, it seems probable that confusion will result. With this in mind I shall now review briefly the opinions expressed by workers in this field as to the alternation of generations in the red algae, and shall venture to offer some suggestions as to the meaning of the rather complicated nuclear life-histories of members of this group.

Oltmanns (58), after a careful study of the development of the cystocarp in four genera, came to the conclusion that the sporogenous cells

constitute a generation similar to the sporophyte of the Archegoniate series. Later (59, ii), he elaborated this conception and expressed the opinion that the sporophyte is of antithetic origin, i.e. that it became gradually intercalated in the life-history by a series of stages of increasing complexity. In those forms in which the tetraspores are borne on distinct plants, he regards the tetraspore-producing plant as a 'facultative gametophyte', which is a result of a process of differentiation similar to that which produced dioecism in many of the Archegoniates. The tetraspores he considers analogous to the gemmae of certain liverworts. Admitting the possibility that a numerical reduction of the chromosomes may take place in the tetrasporangium, he expresses the opinion that the number of chromosomes is not the final test of alternation of generations. 'Ich vermute, die vergleichende Untersuchung des ganzen Entwicklungsganges führt eher zum Ziel, oder aber die Kombination beider Methoden' (59, ii, p. 273).

The work of Wolfe on *Nemalion multifidum* (90), in which he found a numerical reduction of chromosomes just previous to the production of carpospores, furnished a cytological analogy between the cystocarp of the Rhodophyceae and the sporogonium of the Bryophytes, and strengthened the position of Oltmanns.

Yamanouchi (93), after a very complete cytological study of *Poly-siphonia violacea*, reached the following conclusion: 'The sexual plants and the tetrasporic plants present the two distinct phases of an antithetic alternation of generations, with the cystocarp a part of the sporophytic phase' (p. 433). This conclusion is based on the discovery by Yamanouchi that the dividing nuclei of the tetraspore-producing plant throughout its history, as well as those of the sporogenous cells of the cystocarp, show forty chromosomes (the $2x$ number), while the nuclei of the sexual plants show twenty chromosomes (the x number). The number of the chromosomes is reduced in the divisions of the nucleus of the tetraspore-mother-cell; the double number is restored by the union of the nuclei of the gametes. In discussing the origin of the tetraspore, Yamanouchi surmises that in some such form as *Batrachospermum*, in which monospores are borne along with gametes on the sexual plants, reduction may have been suppressed in the formation of the carpospore, 'so that it germinates with the sporophytic number of chromosomes, producing a plant which consequently becomes at once a part of the sporophytic phase. It is quite possible that the first tetraspore-mother-cells corresponded to monospores on the sexual plant, except that they had the double number of chromosomes, since such reproductive cells would very naturally become the seat of the delayed reduction phenomena. The resemblance in general morphology of the tetrasporic plants in the red algae to the sexual plants would be expected because they live under similar environmental conditions' (p. 435).

The views of Oltmanns and Yamanouchi coincide so far as regarding

the sporogenous cells of the cystocarp as belonging to the sporophytic phase of an antithetic alternation of generations. The point of departure lies in the interpretation of the tetraspore-producing plant. Because of its general morphological identity with the gametophyte, Oltmanns regards this as a part of the gametophyte, which has become differentiated for the production of tetraspores. Because of the diploid condition of its nuclei, Yamanouchi regards the tetrasporic plant as a part of the sporophyte, whose resemblance to the gametophyte is stamped on it by 'similar environmental conditions', a view in which Bower (12, p. 81) seems to concur.

For the purposes of the present discussion, I shall assume from the cytological evidence what it will take cultural experiments to prove, namely, that in those red algae in which tetraspores and gametes are regularly formed on separate individuals there is an actual succession of sexual and tetrasporic plants, the reproductive bodies of one kind of plant always producing the other kind of plant.

Yamanouchi's suggestion (93) that the tetrasporic plant may have arisen phylogenetically by the postponement of the phenomena of reduction from the formation of carpospores to the production of asexual spores seems to be rendered probable by what we know of other groups of plants. In the simplest plants which have been investigated from this standpoint, the position of reduction in the life-history seems to be at the first divisions of the fusion nucleus, as is described in *Coleochaete* (Allen, 2), certain desmids (Klebahn, 47), and in *Spirogyra* (Karsten, 45). Beginning with the simple Bryophytes, the familiar Archegoniate series shows a progressive removal of the point of reduction from the point of fusion of the sexual nuclei. These and other examples seem to show that there is a general tendency throughout the plant kingdom to prolong the diploid condition of the nucleus through the greater part of the life-history (see Bower, 12, p. 77).

Nowhere is this more plainly shown than in the Uredineae (Blackman, 8; Blackman and Fraser, 9; Christman, 16, 17). This group is characterized by a succession of phases, or generations, which have been shown by Christman to be morphologically equivalent, though each ends in a distinct form of spore. Now nuclear association, which has been regarded as the equivalent of fertilization in this group, occurs, in all forms in which the aecidial stage is present, in those cells of the mycelium which give rise to the aecidium. The process of numerical reduction of the chromosomes, or, to speak more accurately, of the chromatin, occurs always in the last spore-form preceding the production of aecidia, in the teleutospore when present. The diploid condition, extending from the aecidium to the teleutospore, is lengthened by the intercalation of new phases, which, in some cases, seem to have the power of continuing the diploid generation indefinitely. The haploid generation, from the teleutospore to the binucleate cells at the base of the aecidium, is never lengthened by the inter-

calation of new phases. In other words, in the Uredineae the diploid generation has become prolonged throughout the greater portion of the life-history.

In the red algae it seems likely that a similar postponement of reduction has taken place. In the Nemalionales, which is considered the most primitive group of the Rhodophyceae, the point of reduction is removed from the point of nuclear fusion only by the few cell-generations in the cystocarp (Wolfe on *Nemalion*, 90). In the higher forms, such as the Rhodomelaceae (Yamanouchi on *Polysiphonia*, 93), and the Ceramiaceae (*Griffithsia*), nuclear reduction is separated from nuclear fusion, not only by the cell-generations of the cystocarp, but also by all the divisions of the vegetative cells of the tetrasporic plant. That is, the diploid phase has come to occupy the greater portion of the life-history.

The biological meaning of this apparently general tendency in the evolution of plant structures is hinted at by the experiments of Gerassimow (32). After studying the growth of vegetative cells of *Spirogyra* in which nuclei had been induced to fuse, Gerassimow came to the conclusion that the growth of a cell which has an unusual amount of nuclear material is more vigorous than that of a cell with the usual nuclear content. The cell-wall, the chromatophores, and apparently the protoplasm, grow more vigorously. Such cells divide only after they have reached a size noticeably larger than normal. (See Bot. Gaz., xxxv, 1903, pp. 224-5.)

If, then, the presence of nuclei with the double chromatin content imparts greater vigour to the cell, we should expect to find some evidence of this greater vigour not only in the size, but in the rate of growth of the tetrasporic plants of *Griffithsia*. A comparison of sexual plants with tetrasporic plants does not reveal any constant difference in the size of the resting nuclei or in the size of the cells of the two kinds of individuals. However, a comparison of those cells of the diploid individual which produce sori of tetraspores with the occasional cells of the haploid plant, which form similar structures, shows a difference in size, the cells with the diploid nuclei being larger (p. 672).

More important from this standpoint is the fact that not only in *Griffithsia*, but in red algae generally where tetrasporic and sexual plants occur side by side, the tetrasporic plants are, as a rule, more abundant (p. 640). In *Griffithsia Bornetiana* the number of tetraspores produced is certainly much greater than the number of carpospores, and we should expect, therefore, if the two kinds of spores were equally vigorous, that the number of sexual plants would greatly exceed the number of tetrasporic plants; whereas the reverse is the case. It seems possible that the carpospores have a greater capacity for development than the tetraspores. Cultural experiments along this line are much to be desired.

If the view is correct that a postponement of reduction has occurred in some Rhodophyceae, it is evident that besides the alternation of the

gametophyte (the sexual plant) with the antithetic sporophyte (the sporogenous cells of the cystocarp), there is a succession of homologous phases, inasmuch as a tetrasporic individual regularly succeeds a sexual individual of identical morphology. This latter condition is not paralleled in the Archegoniate series; and since the terms gametophyte and sporophyte have come to have a special significance in connexion with such conditions as are found in the Archegoniates, neither of these terms should be applied to the tetrasporic plants of *Griffithsia* and *Polysiphonia*. The tetrasporic plant has probably been intercalated in the life-history of the red algae, but there is no evidence for the belief that it has been intercalated by gradual integration and differentiation of a simple product of the germination of the zygote, which product was at first unlike the sexual plant and which represents a new departure in the life-history; and the intercalation, by amplification, of an *unlike* phase seems to be the very pith of the theory of antithetic alternation (see Bower, 11; and 12, p. 47; Yamanouchi, 94, p. 310).

According to this view, the tetrasporic plant probably arose, when first produced, with the complete differentiation characteristic of this species. The best evidence for this conclusion is based on the morphological identity of the tetrasporic with the sexual plant. Similar environmental conditions would hardly suffice to produce identity of form in two individuals unless the individuals were from the beginning identical. The tetrasporic plant of a red alga may be said, then, to be homologous with the sexual plant.

That the two phases are homologous is evidenced, not only by their similarity of structure, but by the fact that either seems capable of producing the morphological equivalent of the reproductive structures of the other. It has been known since Bornet first called attention to the fact (10) that in many species of red algae structures resembling tetraspores are occasionally found on the sexual individuals. This phenomenon has been carefully investigated in *Polysiphonia violacea* and *Griffithsia Bornetiana*. In *Polysiphonia*, Yamanouchi (93) found that the development of these tetraspore-like structures ceases at the mother-cell stage; cleavage of the cytoplasm may begin, but normal nuclear division is absent. In *Griffithsia*, the phenomena observed are similar to those noted in *Polysiphonia*, except that by the time the abortive cleavage begins, the nucleus has divided to form several. The cleavage planes have never been observed to reach the centre of the cell, and it is quite evident that tetraspores are not formed, since the whole cell becomes withered and wrinkled, resembling the degenerated tetrasporangia described on p. 668. In one instance, however, a mother-cell was observed in which no trace of cleavage of the cytoplasm was apparent, and in which the number of nuclei had increased to sixteen, the whole structure resembling very much the early stages of germination of a normal spore. It seems quite possible that the tetraspore-mother-cells

borne on the sexual plants sometimes germinate as monospores, though this can be ascertained only by cultivation.

On the other hand, tetrasporic plants may at times produce structures

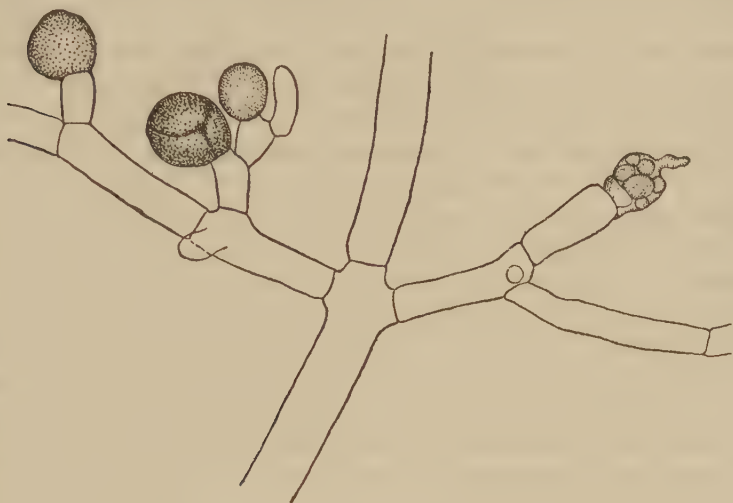


FIG. 1.

morphologically identical with procarys. Text-fig. 1 is taken from a tetrasporic plant of *Spermothamnion Turneri* collected at Wood's Hole in August, 1907, and shows tetraspores and procarys on opposite branches of the same filament. Text-fig. 2 shows a section of the tetrasporangium in which the definitive tetraspores are formed, though not yet separated. Antheridia have not been reported as occurring on *Spermothamnion Turneri* at Wood's Hole, and cystocarys are very rarely produced. Whether functional gametes are ever produced on an individual which bears normal tetraspores is not known.

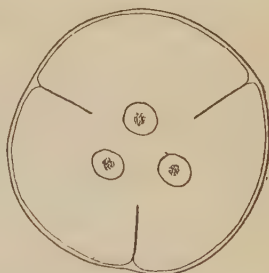


FIG. 2.

In *Spermothamnion Turneri*¹ from Helgoland, Pringsheim states (63, p. 26) that 'Kapselfrüchte, Vierlingsfrüchte und Antheridien normal zusammen auf denselben Exemplaren auftreten'. This case is obviously of so great theoretical interest that I took occasion, in the summer of 1908, to collect this plant at Helgoland. Hundreds of plants were collected, the great majority of which bore only tetraspores. In a few individuals

¹ The species called *Spermothamnion roseolum* by Pringsheim is stated by Professor Kuckuck to be identical with *S. Turneri*. In this connexion I wish to offer my thanks to Professor Kuckuck for the privilege of working at the Biologische Anstalt at Helgoland.

tetraspores were found together with antheridia or procarps, but in no case were tetraspores and cystocarps found on the same plant. Cytological investigation of *Spermothamnion* is much to be desired.

The theory of homologous alternation in the red algae outlined above is almost identical with the view of Pringsheim as to the relations within this group (64). He states (p. 897), 'die Annahme ist nächstliegend, dass bei Florideen und Dictyoteen zwischen Exemplaren mit Kapselfrüchten und Exemplaren mit Vierlingsfrüchten eine Abwechslung besteht.' Pringsheim's view was based, however, on a very different kind of evidence from that brought forward in the present paper.

The evidence at hand seems to justify the following conclusions:

1. There is in *Griffithsia* an antithetic alternation of generations, the gametophyte being represented by the sexual plants, the sporophyte by the sporogenous cells of the cystocarp.

2. In addition to this, there is a regular succession of tetrasporic individuals and sexual individuals. The tetrasporic individuals resemble the sporophyte in number of chromosomes; they resemble the gametophyte in morphological differentiation. They are to be considered as a phase of an homologous alternation of generations, not the equivalent, wholly or in part, of the sporophyte of Archegoniates.

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EXPLANATION OF PLATES XLIX-LIII.

Illustrating Dr. Lewis's paper on *Griffithsia Bornetiana*.

Lettering of figures: *a.n.*, auxiliary nucleus; *aux.*, auxiliary cell; *b.c.*, basal cell; *carp.*, carpgonium or carpogenic branch; *c.c.*, central cell; *f.n.*, fusion nucleus; *p.c.*, peripheral cell; *pl.*, placenta; *pl.c.*, placental cell; *sp.c.*, sporogenous cell.

PLATE XLIX (Figs. 1-26).

- Fig. 1. Small specimen, female plant. × 2.
- Fig. 2. Portion of male plant, showing caps of antheridia over the tips of the terminal cells, and hairs. × 30.
- Fig. 3. Portion of female plant with cystocarps. × 30.
- Fig. 4. Portion of tetrasporic plant with tetraspores. × 30.
- Fig. 5. Optical section of the wall of a single cell, on the left from near the apex, on the right from the base, showing characteristic lamination. × 1800.
- Fig. 6. Longitudinal section through an intercellular connexion. × 700.
- Fig. 7. Section showing accumulation of nuclei and cytoplasm on the wall. × 1200.
- Fig. 8. Transverse section through an intercellular connexion, showing the spore surrounded by nuclei; among the nuclei are deeply staining balls of proteid. × 700.
- Fig. 9. Surface view of an apical cell, in which all the nuclei are undergoing division. × 500.
- Fig. 10. Resting nucleus from a tetrasporic plant; around it are chromatophores. × 1800.
- Figs. 11-26. Stages in mitosis of nuclei from tetrasporic plant. All × 3600.
- Figs. 11-18, prophase; 19, metaphase showing spindle, chromosomes, and kinoplasmic caps; 20, polar view of equatorial plate; 21-23, anaphases; 24-26, telophases.

PLATE L (Figs. 27-54).

Figs. 27-31. Stages in mitosis of nuclei from male plant. $\times 3600$.

Figs. 27, 28, prophase; 29, 30, polar view of equatorial plate at metaphase; 31, anaphase.

Fig. 32. Surface view of chromatophores, showing arrangement; one nucleus in the field. $\times 1200$.

Fig. 33. Dividing chromatophores. $\times 3600$.

Figs. 34-41. Stages in cell-division, longitudinal sections. $\times 330$.

Fig. 34, accumulation of protoplasm in tip of the cell; 35, formation of dome-shaped membrane; 36, beginning of vacuole; 37, slightly older stage; 38, small 2-celled lateral branch, with neighbouring hair; 39, beginning of formation of ring-shaped constriction; 40, 41, stages in ingrowth of ring-shaped partition.

Fig. 42. Hair-like organs of different ages side by side. $\times 330$.

Fig. 43. Same after older hair has elongated. $\times 125$.

Fig. 44. Three rhizoids on single cell, longest one toward base of cell. $\times 30$.

Fig. 45. Tendril-like rhizoid twining around support. $\times 45$.

Fig. 46. Rhizoids connecting neighbouring cells. $\times 15$.

Figs. 47-50. Stages in regeneration, showing how continuity is restored in filament upon the death of a cell. $\times 30$.

Fig. 51. Young attaching disk, attaching cells shaded. $\times 30$.

Fig. 52. Older stage of same. $\times 15$.

Fig. 53. Surface view of young antheridial branch, with hair. Antheridial papillae cover tip of apical cell. $\times 130$.

Fig. 54. Longitudinal section of apical cell bearing antheridial papillae. $\times 350$.

PLATE LI (Figs. 55-89).

Fig. 55. Antheridial papillae cut off from mother-cell. $\times 1200$.

Figs. 56, 57. Division of antheridial papillae. $\times 1800$.

Fig. 58. Antheridial papillae lying in swollen wall of mother-cell. $\times 1800$.

Fig. 59. Same, after division of the papillae. $\times 350$.

Figs. 60, 61. Stages in formation of antheridial filaments. $\times 1800$.

Figs. 62, 63. Antheridial filaments with mature spermatia. $\times 1200$.

Figs. 64, 65. Formation of new antheridial branches after terminal cell has ceased to produce antheridia. $\times 20$.

Fig. 66. Surface view, to show 2-celled procarp; the small apical cell has been pushed to one side. $\times 100$.

Fig. 67. Longitudinal section of similar stage. $\times 500$.

Fig. 68. Longitudinal section of 3-celled procarpic branch. $\times 500$.

Fig. 69. Surface view, showing first peripheral cell. $\times 500$.

Fig. 70. Surface view, showing second peripheral cell. $\times 500$.

Fig. 71. Surface view of later stage; 3-celled carpogenic branch shaded. $\times 500$.

Fig. 72. Procarpic branch metamorphosed into vegetative shoot; on either side are ordinary vegetative branches. $\times 150$.

Fig. 73. Almost mature procarp; carpogenic branch shaded. $\times 500$.

Fig. 74. Section of very young carpogonium. $\times 1800$.

Fig. 75. Mature procarp; spermatium attached to tip of trichogyne. $\times 500$.

Fig. 76. Longitudinal section of procarp of same age as in Fig. 71. $\times 500$.

Fig. 77. Neighbouring longitudinal sections of mature procarp; two spermatia attached to trichogyne; two male nuclei in trichogyne. $\times 500$.

Fig. 78. *a.* Longitudinal section through trichogyne, carpogonium, and auxiliary cell. $\times 500$.
b. Tip of same trichogyne. $\times 1800$.

Fig. 79. Auxiliary cell and carpogonium in longitudinal section; male nucleus entering carpogonium. $\times 3600$.

Fig. 80. Surface view from above of young cystocarp in which trichogyne has begun to wither. $\times 500$.

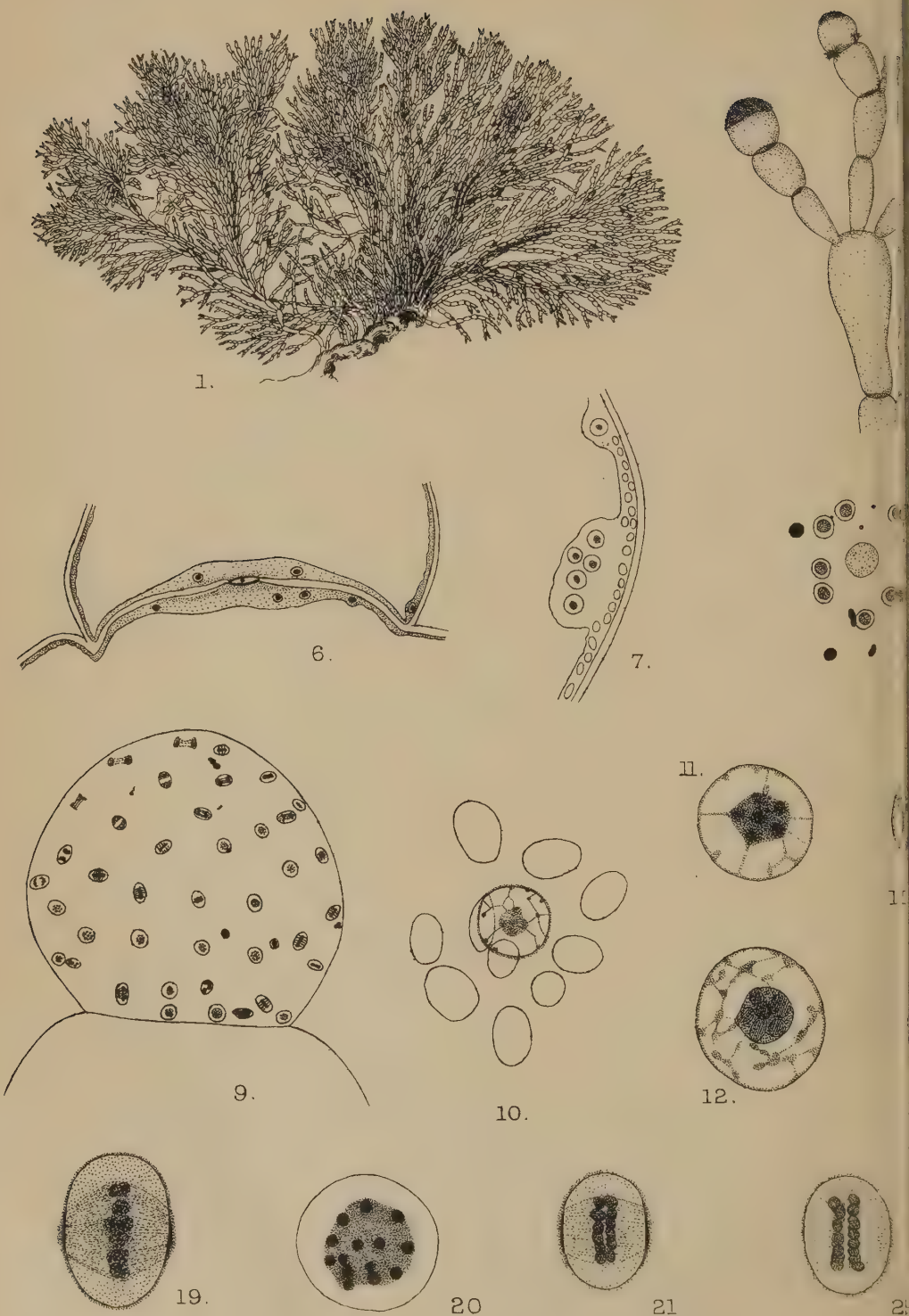
- Fig. 81. Longitudinal section; fusion nucleus present in auxiliary cell. $\times 500$.
 Fig. 82. Surface view of young cystocarp showing withered carpogenic branch falling off as a whole. $\times 500$.
 Fig. 83. Longitudinal section showing two fusion nuclei in auxiliary cell. $\times 500$.
 Fig. 84. Longitudinal section showing auxiliary cell divided into two, the upper cell the placental cell. $\times 500$.
 Fig. 85. Longitudinal section of cystocarp in which sporogenous lobes have been formed from placental cell. $\times 500$.
 Fig. 86. Formation of sporogenous lobes from auxiliary as well as placental cell. $\times 500$.
 Fig. 87. Fusion of cells in centre of cystocarp; central cell, placental cell, auxiliary, and first peripheral cell are shown taking part in the fusion. $\times 500$.
 Fig. 88. Degenerating nuclei from the lower part of the placenta. $\times 3600$.
 Fig. 89. Division of sporogenous lobe; crescent-shaped cell is cut off on outside. $\times 500$.

PLATE LII (Figs. 90-128).

- Figs. 90-93. Sections of sporogenous lobes, showing mode of division. $\times 500$.
 Fig. 94. Mitosis in sporogenous lobe, and division by constriction of cytoplasm. $\times 1200$.
 Fig. 95. Surface view of cystocarp producing mature spores; sporogenous lobes of various ages are shown. $\times 150$.
 Fig. 96. Section of mature carpospore. $\times 700$.
 Fig. 97. Spindle and polar view of equatorial plate from mitoses in sporogenous lobes. $\times 1800$.
 Figs. 98, 99. Tetrasporic papillae pushing out through wall of vegetative mother-cell. $\times 850$.
 Figs. 100, 101. Tetrasporic papillae cut off from mother-cell. $\times 850$.
 Figs. 102-4. Division of primary tetrasporic cell. $\times 850$.
 Fig. 105. Basal cell and tetraspore-mother-cell. $\times 850$.
 Fig. 106. Second division of basal cell. $\times 850$.
 Fig. 107. Basal cell with two tetraspore-mother-cells; in one three definitive spore nuclei are visible. $\times 850$.
 Fig. 108. Basal cell with three tetraspore-mother-cells. $\times 500$.
 Fig. 109. Connexion of basal cell with tetraspore-mother-cell. $\times 3600$.
 Fig. 110. Division of mother-cell into spores; basal cell shows tubular process. $\times 500$.
 Fig. 111. Mother-cell; nucleus shows 14 granules. $\times 850$.
 Fig. 112. Chromatin granules fewer in number and irregular in outline; nuclear (chromidial) material is shown passing out into cytoplasm. $\times 850$.
 Fig. 113. About same stage; chromatin granules show indications of tetrad grouping. $\times 850$.
 Fig. 114. Nucleus of tetraspore-mother-cell in prophase; chromosomes scattered through nuclear cavity. $\times 1800$.
 Fig. 115. Same stage. $\times 1800$.
 Fig. 116. Longitudinal section of tetraspore-mother-cell; nucleus in metaphase. $\times 1800$.
 Fig. 117. Metaphase, first mitosis. $\times 1800$.
 Fig. 118. Prophase, first mitosis; chromosomes gathering in centre of nuclear cavity. $\times 1800$.
 Fig. 119. Prophase; chromosomes seem to be grouped in pairs. $\times 1800$.
 Fig. 120. Polar view of equatorial plate showing fourteen chromosomes. $\times 1800$.
 Fig. 121. Anaphase, showing separation of two groups of chromosomes (from neighbouring sections). $\times 1800$.
 Fig. 122. Telophase; nuclear cavity dividing into two. $\times 1200$.
 Fig. 123. Two daughter-nuclei in mother-cell. $\times 1800$.
 Fig. 124. Prophase, second mitosis. $\times 3600$.
 Fig. 125. Longitudinal section, tetraspore-mother-cell, upper nucleus showing spindle at metaphase, lower showing polar view of equatorial plate with seven chromosomes. $\times 1800$.
 Fig. 126. Telophase, second mitosis. $\times 1200$.
 Fig. 127. Three definitive spore nuclei visible in mother-cell; into lowest food material is passing from basal cell. $\times 850$.
 Fig. 128. Portion of wall of tetrasporangium showing beginning of cleavage. $\times 3600$.

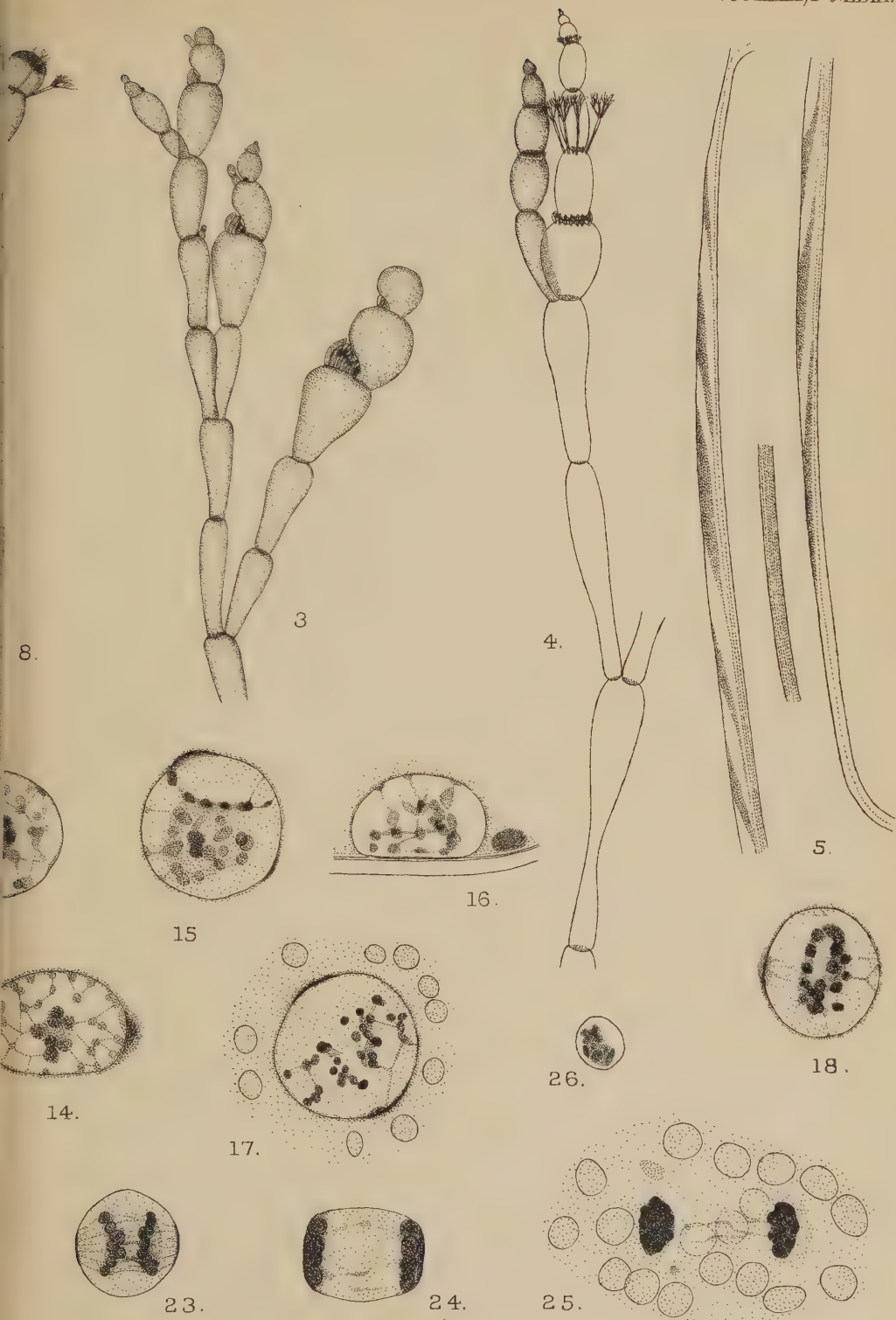
PLATE LIII (Figs. 129-58).

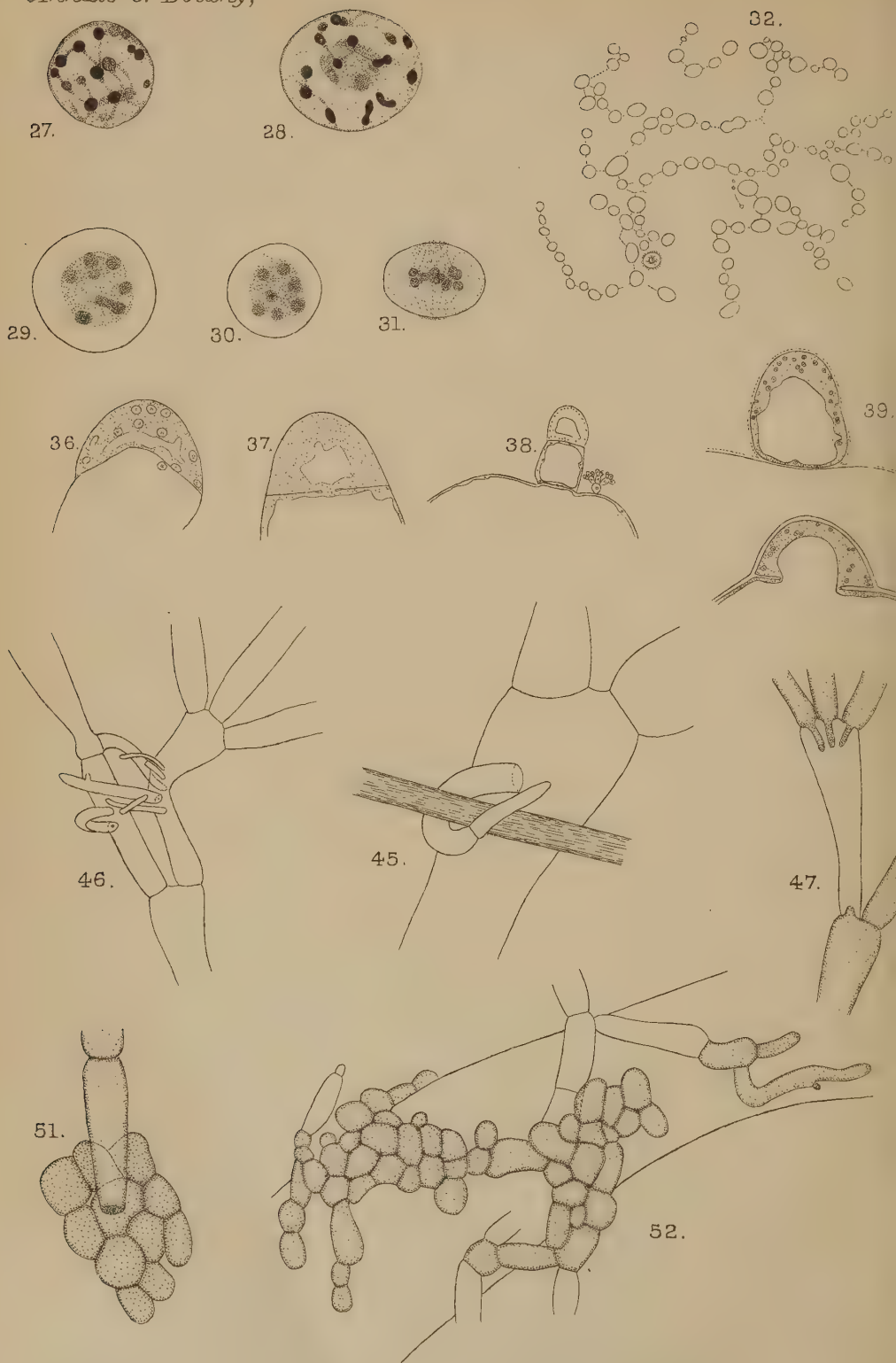
- Fig. 129. Partitions about one-third of the way to centre of tetrasporangium. $\times 850$.
 Fig. 130. Later stage, partitions double, chromatophores visible around periphery. $\times 850$.
 Fig. 131. Partitions almost reach to centre of tetrasporangium; spores nearly separated.
 $\times 850$.
 Fig. 132. Spores completely separated, still enclosed by gelatinized wall of tetrasporangium.
 $\times 330$.
 Fig. 133. Diagrammatic longitudinal section showing mode of occurrence of tetrasporic structures. $\times 30$.
 Figs. 134-8. Tetraspore-like structures from male plant. $\times 850$.
 Fig. 134. Basal cell with two tetraspore-mother-cells (cf. Fig. 108).
 Fig. 135. Later stage, only one tetraspore-mother-cell present.
 Fig. 136. Basal cell with tubular outgrowth (cf. Fig. 110).
 Fig. 137. Tetraspore-mother-cell showing eight nuclei; cleavage of cytoplasm has begun.
 Fig. 138. Sixteen nuclei present in mother-cell, no trace of cleavage.
 Fig. 139. Vegetative cell accidentally isolated has produced a rhizoid from basal end. $\times 100$.
 Fig. 140. Later stage showing young plant developed from similar isolated cell. $\times 15$.
 Fig. 141. Binucleate sporeling. $\times 850$.
 Fig. 142. Eight-nucleate sporeling, optical section. $\times 500$.
 Fig. 143. Section of sixteen-nucleate sporeling. $\times 850$.
 Fig. 144. Sporeling has begun to elongate without division. $\times 500$.
 Fig. 145. Two-celled sporeling. $\times 850$.
 Fig. 146. Three-celled sporeling. $\times 850$.
 Fig. 147. Same, surface view. $\times 30$.
 Fig. 148. Three-celled sporeling after elongation, attached to filament of *Lomentaria*. $\times 100$.
 Fig. 149. Similar stage, showing difference in relative size of the cells. $\times 100$.
 Fig. 150. Sporeling of same age growing on glass; basal cell very long. $\times 100$.
 Fig. 151. Similar sporeling after basal cell has reached a suitable substratum. $\times 100$.
 Fig. 152. Section showing basal cell of sporeling applied to surface of *Champia*. $\times 100$.
 Fig. 153. Section showing basal cell penetrating tissues of *Champia*. $\times 100$.
 Figs. 154-6. Development of attaching disk from basal cell. $\times 30$.
 Fig. 157. Four-celled sporeling; basal cell branched. $\times 30$.
 Fig. 158. Sporeling beginning to branch. $\times 30$.



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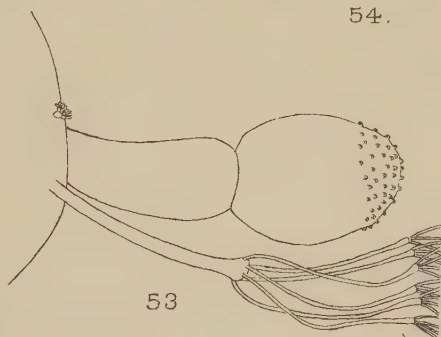
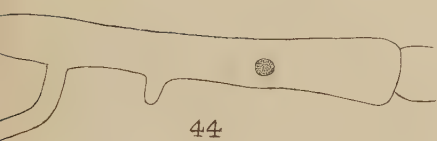
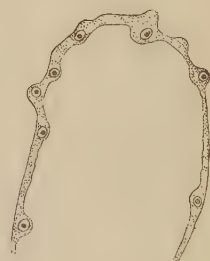
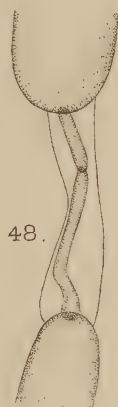
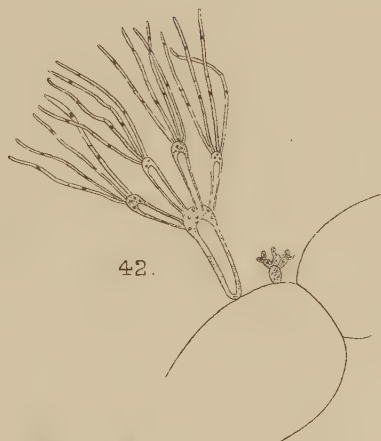
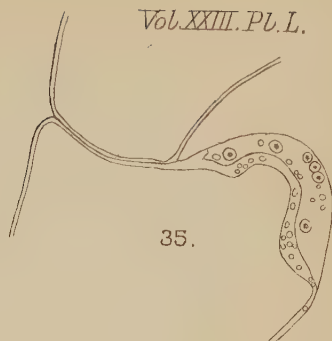
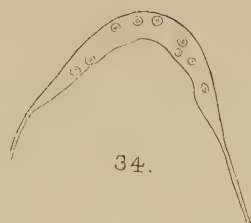
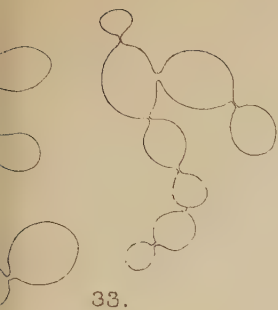
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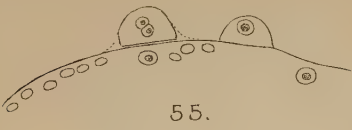




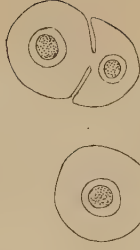
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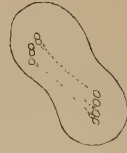




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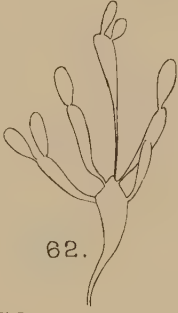
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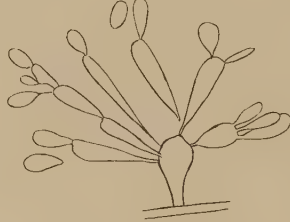
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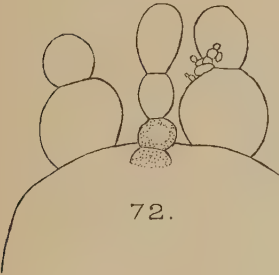
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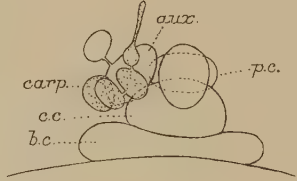
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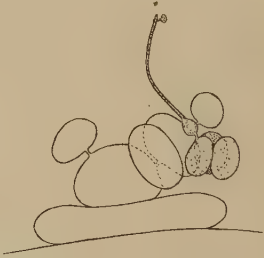
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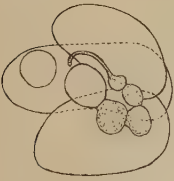
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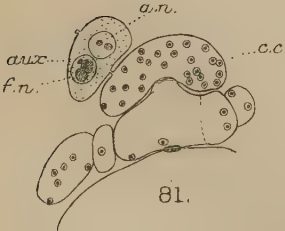
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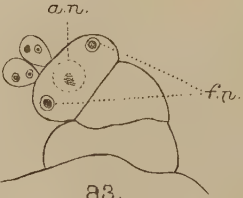
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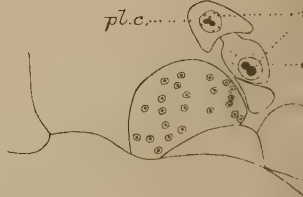
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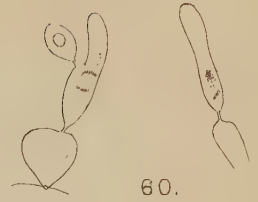


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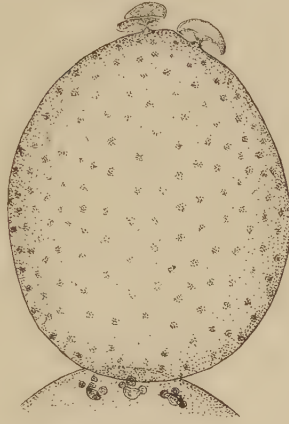
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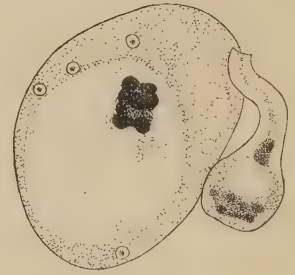
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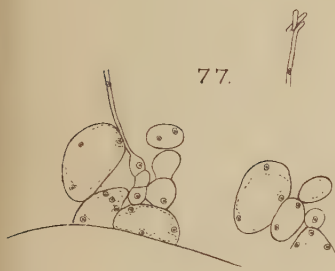
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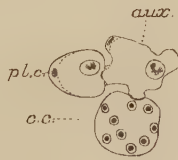
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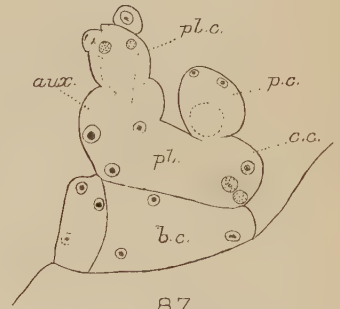
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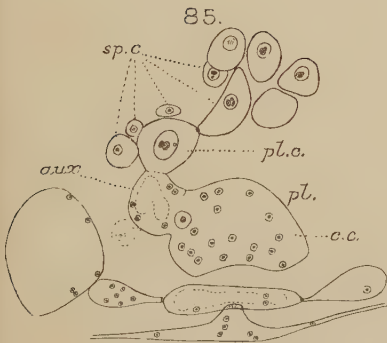
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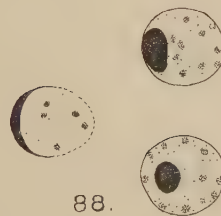
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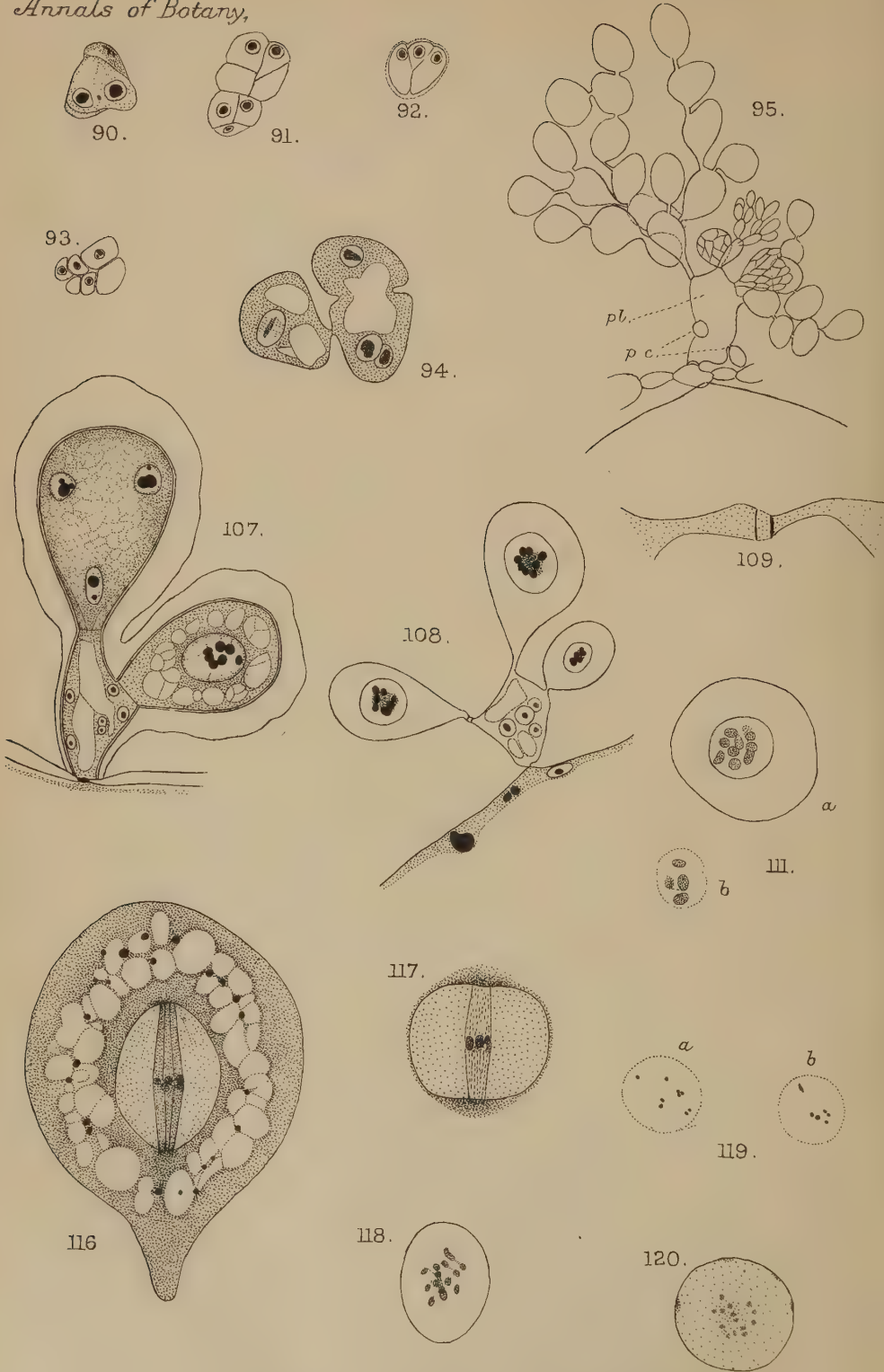
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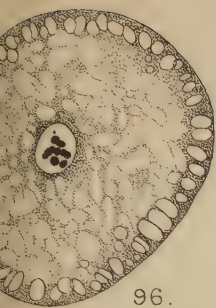
88.



89.



I.F.L. del.



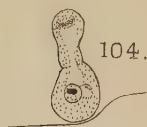
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101.



104.



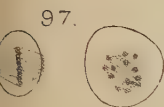
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97.



100.



103.



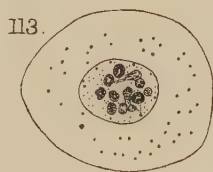
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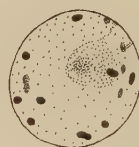
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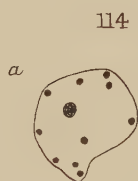
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115



114

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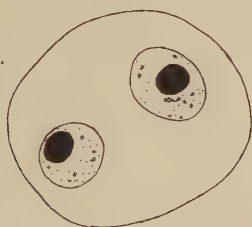


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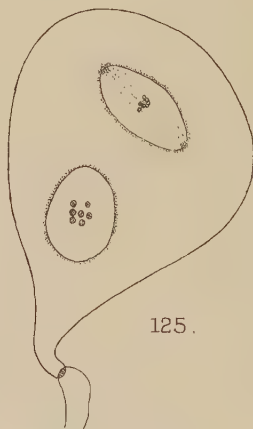


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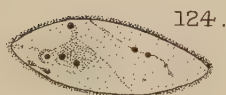
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125.



124.



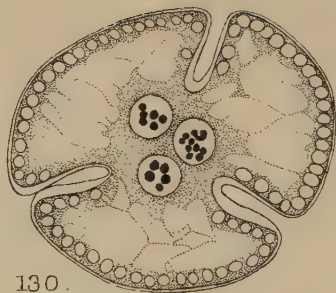
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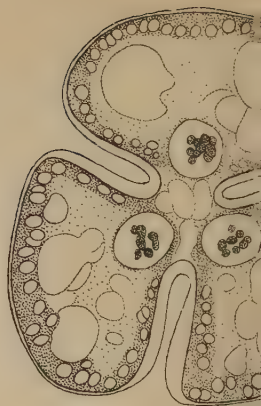
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130.



131.



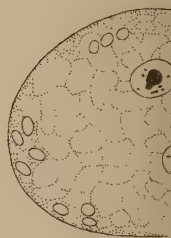
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139.



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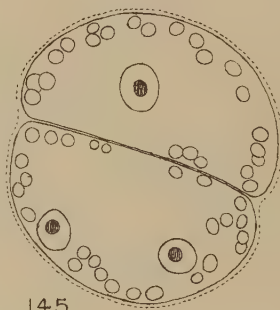
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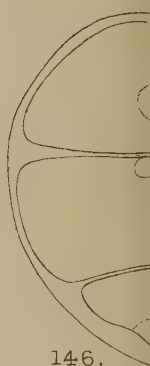
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144.



145.



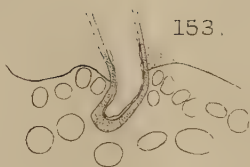
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152.



150



153.



154.



155.

I.F.L. del



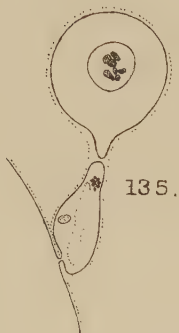
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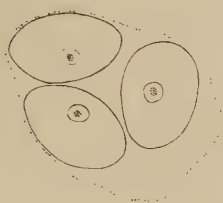
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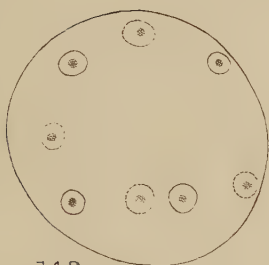
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137.



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142.



147.



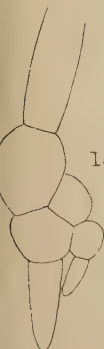
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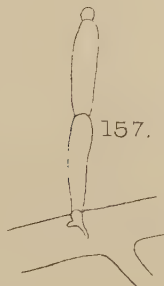
149.



151.



156.



157.



158.

VITA.

Ivey Foreman Lewis was born in Raleigh, N. C., August 31, 1882. He entered the University of North Carolina in 1898, and was graduated in 1902 with the degree of A. B. In 1903 he acted as assistant in Biology at this institution, and received the degree of M. S. He attended the Johns Hopkins University during 1903-1905 and 1906-1908, making Botany his principal subject of study, Zoology his first subordinate and Physiology his second subordinate. In 1905-1906 was Acting-Professor of Biology in Randolph-Macon College, Ashland, Va., and was elected Professor in 1907, with one year's leave of absence.

The subject of this sketch wishes to express here his grateful appreciation of the interest shown and the assistance rendered him during his university course by Professors Brooks, Andrews, Grave, and Howell and especially to express his thanks to Professor D. S. Johnson for his stimulating advice and criticism, and for his unflagging interest and constant assistance.

